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DRINKING WATER HEALTH DOCUMENT ON TOLUENE

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DRINKING WATER CRITERIA DOCUMENT FOR TOLUENE

Prepared for

OFFICE OF DRINKING WATER

Prepared by

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The Office of Drinking Water (ODW), Environmental Protection Agency has prepared a Drinking Water Criteria Document on Toluene. This Criteria Document is an extensive review of the following topics:

- Physical chemical properties of Toluene
- Toxicokinetics and human exposure to Toluene
- Health Effects of Toluene in humans and animals
- Mechanisms of toxicological effect of Toluene
- Quantification of toxicological effects of Toluene

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1984; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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LIST OF ABBREVIATIONS

BTX	Benzene-toluene-xylene mixture
BUN	Blood urea nitrogen
bw	Body weight
CNS	Central nervous system
DNA	Deoxyribonucleic acid
DWEL	Drinking water equivalent level
EEG	Electroencephalogram
GI	Gastrointestinal
HA	Health advisory
i.p.	Intraperitoneal
i.v.	Intravenous
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
LDH	Lactate dehydrogenase
MFO	Mixed function oxidase
MTD	Maximum tolerated dose
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
ppm	Parts per million
RBC	Red blood cell
RfD	Reference dose
s.c.	Subcutaneous
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvate transaminase
TLV	Threshold limit value
TWA	Time-weighted average
W	Watts
WBC	White blood cell

I. SUMMARY

Toluene is a relatively volatile organic solvent with a low water solubility, so that in aqueous solution it would be expected to evaporate into the environmental air. Because of its predisposition to exist as a vapor in the environment, most of toluene exposure in humans is by inhalation. Experimental studies have reflected this in an attempt to study realistic toluene exposure. Few data are available regarding oral toluene exposure in comparison with exposure by inhalation.

Absorption of toluene is rapid by inhalation or from the GI tract. Absorption from the GI tract appears to be complete, while ~50% of the inhaled toluene is absorbed through the lungs. Cutaneous absorption of toluene occurs to some extent, but may represent ~1% of that absorbed by the lungs when there is exposure to toluene vapor. Dermal absorption of liquid toluene may be quite efficient (14-23 mg/cm^2/hour). However, because toluene readily evaporates, it is unlikely that absorption will occur by this exposure route. Distribution of toluene following absorption occurs throughout the body with accumulation in adipose and other tissues with high lipid content. Bioaccumulation of toluene is unlikely, since retention time of toluene is usually considered to be ~24 hours. Toluene is metabolized rapidly, predominantly in the liver, by oxidation and conjugation to hippuric acid (60-70% of absorbed dose) and benzoyl glucuronide (10-20% of dose). Ring hydroxylation to cresols is a minor metabolic pathway for toluene. Elimination of unchanged toluene by the lungs or skin accounts for 10-20% of the absorbed dose. Excretion of toluene metabolites is rapid, being complete within 8-24 hours of exposure, depending on the dose. The toxicokinetic profile of toluene in humans is not notably different from that in laboratory animals.

The LD_{50} of toluene administered orally is between 5300 and 7500 mg/kg in mature rats. The first observable sign of toluene toxicity is that of CNS depression occurring at 2000 mg toluene/kg in rats given a single oral dose. A similar effect, loss of coordination, occurs in rats exposed by inhalation to 1250 ppm toluene for 18-20 hours. Histological changes in the lungs, liver and kidneys are evident at comparable dose levels following acute exposure.

Subchronic oral administration of toluene at 590 mg/kg/day 5 days/week for 193 days caused no observable effects on female Wistar rats. Inhalation of toluene at 200 ppm (754 mg/m³) for 7 hours/day, 5 days/week over a 6-week period caused slight pulmonary irritation and the development of casts within the renal tubules of treated rats. Generally, animals exposed to toluene at or near 100 ppm show no notable effects. In the only chronic study available, exposure to 30, 100 or 300 ppm toluene by inhalation for 6 hours/day, 5 days/week for 106 weeks caused no dose-related toxic effects in F344 rats. Toxic effects following prolonged exposure are similar to those seen following acute exposure, predominantly on the CNS, liver, kidneys and lungs.

Toluene has not been adequately tested for carcinogenicity. In the only available chronic bioassay, no difference was detected in the incidence of tumors or proliferative masses between control rats and rats treated at 30, 100 or 300 ppm toluene (same inhalation dose schedule as above). Mutagenicity assays using microbial species and rat and human blood cell assays have not shown toluene to be a mutagen in the presence or absence of an activation system. Toluene administered by gavage to CD-1 mice at

260 mg/kg/day on days 6-15 of gestation caused embryo death, while higher doses (430 or 870 mg/kg/day) additionally caused fetal and maternal toxicity and cleft palates. These data were reported in an abstract. A complete report with additional details was not available. The data are therefore viewed circumspectly. In three teratogenicity studies in which toluene was administered to rats or mice by inhalation, no dose-related abnormalities were reported, though it was suggested that skeletal maturation was delayed in exposed fetuses.

In humans, the major acute effects of toluene are CNS dysfunction and narcosis. Occupational exposure to toluene at levels higher than the TLV (100 ppm) can also lead to effects on the nervous system. Experimental exposures in humans show that toluene at 100 ppm for 8 hours causes few, if any, effects. Higher levels can cause lassitude, nausea, fatigue, confusion, incoordination and muscular weakness. Exposure at 300 and 700 ppm for 20 minutes causes decreased reaction time and perceptual speed, respectively. Chronic exposure to toluene is associated with disturbances in nervous or neuromuscular function. Sensorimotor speed and psychomotor skills based on performance testing reportedly have been reduced in some toluene-exposed workers. Cerebral and cerebellar effects, including ataxia, tremors and speech, hearing and vision impairments, also have been occasionally reported in long-term abusers of toluene. Hematological effects formerly associated with toluene have since been attributed to benzene contamination of technical-grade toluene. Hepatomegaly and impaired liver function have been reported in some humans chronically exposed to toluene. Abnormalities in renal function, including decreased hydrogen ion excretion, are being reported more frequently as caused by toluene in exposed humans. Groups considered to be at highest risk of toluene toxicity are toluene-exposed workers, pregnant women, cigarette smokers and abusers.

The mechanism of toluene toxicity is not clearly understood. Pulmonary and renal effects may be due to solvation of structural lipids in the cells, causing changes in cell permeability. Effects on the CNS may be due to high toxicant levels in the myelin or the resulting cell membrane changes, each causing altered axonal function. The toxicity of toluene may be increased by concomitant exposures to benzene and other solvents that result in inhibition of toluene metabolism.

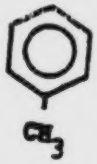
The noncarcinogenic effects of toluene in laboratory animals and humans have been utilized for derivation of HAs and a DWEL. The 1-day HA value, based on a human 8-hour NOAEL, was 18 mg/ℓ for a 10 kg child. Studies appropriate for the derivation of the 10-day and longer-term HAs were not located in the available literature. It was therefore recommended that as a conservative estimate of exposure the RfD be used to derive the 10-day HA and the longer-term HA for a 10 kg child. The proposed 10-day and longer-term HA for a 10 kg child is 3 mg/ℓ. The longer-term HA for a 70 kg adult adopted the DWEL of 10 mg/ℓ as a conservative estimate of exposure. The DWEL was derived from a NOAEL for male and female F344 rats exposed to toluene by inhalation at 300 ppm (6 hours/day, 5 days/week) for 106 weeks. The resultant DWEL for toluene was 10 mg/ℓ. The lower 10-day HA value may be due to the toxicokinetic nature of toluene and the method by which this HA was derived.

Quantification of carcinogenic effects could not be performed because of insufficient data. Applying the criteria described in EPA's guidelines for assessment of carcinogenic risk, toluene may be classified as Group D: Not classified as to human carcinogenicity. This classification reflects the inadequate nature of the available data thus preventing the development of a more definite statement regarding carcinogenic potential.

II. PHYSICAL AND CHEMICAL PROPERTIES

Structure and Identification

The structure, molecular formula, molecular weight, Chemical Abstracts Service (CAS) Registry number, Registry of Toxic Effects of Chemical Substances (RTECS) number and common synonyms for toluene are listed below:



Molecular formula: C_7H_8

Molecular weight: 92.15

CAS number: 108-88-3

RTECS number: XS5250000

Synonyms: Methylbenzene, Phenylmethane, Toluol, methacide, methylbenzol

Physical and Chemical Properties

Toluene is a clear, colorless liquid with a pungent, benzene-like odor. The vapor pressure of toluene is relatively high. Toluene is soluble at ~535 mg/ℓ in fresh water but miscible with most organic solvents. A more complete summary of the physical properties for toluene is listed in Table II-1.

Based on both the physical properties of toluene and the testing data available, the dominant aquatic environmental process for toluene will be volatilization; however, biodegradation and sorption also occur. Chemical processes (and photochemical processes since toluene does not absorb light above 290 nm) appear unlikely to compete significantly with volatilization and biodegradation; a possible exception might be chlorination.

TABLE II-1

Physical Properties of Toluene

Physical Property	Description	Reference
Boiling point	110.6°C	Weast, 1977
Melting point	-94.9°C	Weast, 1977
Density	0.8669 g/mℓ at 20°C	Weast, 1977
Specific gravity	0.8623 at 15.6°C	Cier, 1969
Vapor pressure	28.7 mm Hg at 25°C	Weast, 1977
Water solubility: freshwater sea water	 535 mg/ℓ 379 mg/ℓ	Sutton and Calder, 1975
Log Octanol/Water partition coefficient	2.69	Tute, 1971
Lipid solubility	miscible with most organic solvents	Weast, 1977
Conversion factors	1 ppm = 3.77 mg/m³ 1 mg/m³ = 0.265 ppm	
Taste and odor threshold in water	1 mg/ℓ	Zoeteman et al., 1971
	0.04 mg/ℓ (odor)	Amoore and Hautala, 1983

Toluene in water can be oxidized by singlet oxygen, alkyl peroxy radical ($RO_2\cdot$), alkoxy radical ($RO\cdot$) or hydroxyl radical ($\cdot OH$) (Mabey et al., 1981). For toluene, the most rapid oxidation process is a peroxy radical oxidation (Mabey et al., 1981). Based on experimental evidence and structure, Mabey et al. (1981) calculated a peroxy radical rate constant of 144 M^{-1} hr^{-1}. When this value was used in an EXAMS model, the half-lives for a pond, eutrophic lake, oligotrophic lake and river were 1050, 707, 711 and 707 years, respectively.

Carlson et al. (1975) observed that toluene may form small amounts of chlorine-substituted products during chlorination under conditions used for water renovation. The extent of chlorination increased with the decrease in pH and increase in contact time. At a water temperature of 25°C and a chlorine concentration of 7×10^{-4} M, the percent chlorine uptake was determined to be 11.1 and 29% at a water pH of 3 and 7, respectively. With other conditions remaining the same, no chlorine uptake was observed at a water pH of 10.1.

Chlorination of toluene under ultraviolet light (i.e., formation of $Cl\cdot$) yields several methyl substitution products (Cier, 1969). Hydrolysis of one of the products, benzalchloride, yields benzaldehyde (Gait, 1967). These reactions may have significance with respect to drinking water chlorination since benzaldehyde and benzoic acid (an oxidation product of benzaldehyde) have been detected in drinking water (U.S. EPA, 1979).

A number of reports detailing the biodegradation of toluene in soil, sewage and wastewater were located in the available literature; however, no reports were found in which toluene degradation in water was studied. Most of the studies showed that toluene was biodegraded almost completely within 40 days (Marion and Malaney, 1963; Lutin et al., 1965; Price et al., 1974; Bridie et al., 1979; Patterson and Kodukala, 1981; Tabak et al., 1981). An EXAMS model using the water column biolysis rate constant (1×10^{-7} mL $cell^{-1}$ hr^{-1}) developed by Mabey et al. (1981) yielded a half-life for toluene in a pond, eutrophic or oligotrophic lakes and rivers of 70, 350, 47, 400 and 14 hours, respectively.

Volatilization is the most important transport process for toluene in the environment. An EXAMS model of the fate of toluene in water using a Henry's law constant of 6.66×10^{-3} atm m^3 mol^{-1}, a vapor pressure of 28.7 torr and a reaeration rate ratio of 0.526 (Mabey et al., 1981) suggested volatilization as the most important removal process. Half-lives for toluene in pond, eutrophic lake, oligotrophic lake and river were 74, 234, 268 and 49 hours, respectively. Volatilization for all environments except the river accounted for >58% of the toluene loss.

Uses

Most of the toluene produced remains as a benzene-toluene-xylene (BTX) mixture for use in gasoline. Isolated toluene is used for various purposes. Table II-2 lists the consumption and primary uses of Toluene. The primary use of isolated toluene is in the production of benzene. The second and third largest uses are back-blending into gasoline (which increases octane ratings) and as solvents for paint and coatings, respectively. Adhesives, inks and pharmaceuticals also use significant amounts of isolated toluene. Toluene can also be used as a raw material in the production of

TABLE II-2

Consumption of Isolated and Non-Isolated Toluene in Different Usages*

Usage	Amount Used/Year (10^6 Kg)	% of total Use in Each Cagetory
Non-isolated:		
Gasoline as BTX	27,293	100
Isolated:		
Benzene dealkylation	1,675	40.2
Gasoline back-blending	1,465	35.1
Solvent for paint and coatings	263	6.3
Solvent for adhesives, inks, and pharmaceuticals	132	3.2
Toluene diisocyanate	200	4.8
Xylene	98	2.4
Benzoic acid	65	1.6
Benzyl chloride	36	0.9
Vinyl toluene	25	0.6
Miscellaneous others	39	0.9
Net export	172	4.1
Total	4,170	100.1

*Source: U.S. EPA, 1983

toluene diisocyanate, benzyl chloride, benzoic acid, xylene and vinyl toluene (U.S. EPA 1983).

Summary

Toluene is a colorless, clear liquid with a pungent odor. Toluene has a molecular weight of 92.15, a vapor pressure of 28.7 torr, a freshwater solubility of 535 mg/ℓ and a log octanol/water partition coefficient of 2.69 (Tute, 1971; Sutton and Calder, 1975; Weast, 1977). It is highly volatile and volatilization is likely to be the dominant removal process from ambient waters (based on an EXAMS model using data from Mabey et al., 1981). Biodegradation is also an important removal process; however, no biodegradation tests were found in the available literature that used ambient waters as an inoculum source; consequently, its relative importance is difficult to estimate (Bridie et al., 1979; Tabak et al., 1981). Most chemical processes such as photolysis and oxidation are not important, with the exception of the oxidation of toluene to benzaldehyde during water chlorination (Gait, 1967; Cier, 1969).

The primary uses of isolated toluene include the production of benzene, back-blending in gasoline, and solvents for paint and coatings. Toluene is also used in adhesives, inks and pharmaceuticals.

III. TOXICOKINETICS

Absorption

Based on animal metabolic studies, toluene appears to be almost completely absorbed from the GI tract (Smith et al., 1954; El Masry et al., 1956). An aqueous solution of purified toluene (347 mg/kg bw) was administered by stomach tube to rabbits (strain not specified) and the elimination of toluene or its metabolites was measured (Smith et al., 1954). Pulmonary exhalation of unchanged toluene accounted for 18-19% of the administered dose, while urinary benzoic acid and hippuric acid accounted for up to 80% of the oral dose. El Masry et al. (1956) reported that urinary excretion of hippuric acid following oral administration of 3.0 mmol/kg (276 mg/kg bw) toluene accounted for 74% of the administered dose in Chinchilla rabbits. Taken together, the data from these two studies (Smith et al., 1954; El Masry et al., 1956) conducted in the same laboratory indicate that the percentage of an oral dose (276-347 mg/kg bw) recovered as urinary metabolites or in expired air was nearly 100% in treated rabbits. In male rats absorption from the GI tract also appeared relatively rapid, with maximal blood-toluene levels being reached within 2 hours after gastric intubation with 100 μℓ 4-H^3-toluene in peanut oil (Pyykko et al., 1977). These findings are complicated by the fact that the authors did not distinguish between parent compound and metabolites. The use of peanut oil as a vehicle may have delayed the actual absorption rate.

The most common route of human exposure to toluene is by inhalation. Absorption data by this route in animals and humans are abundant. In dogs exposed for 8 hours to toluene at 200, 400 or 600 ppm (750, 1500 or 2250 mg/m^3), venous blood concentration increased in a dose-related fashion (von Oettingen et al., 1942a). Uptake of toluene vapor after 1-2 minute exposures was ~90% in dogs (Egle and Gochberg, 1976). Increases in ventilatory rate (5-40 inhalations/minute), tidal volume (114-227 mℓ/breath) and toluene concentration (from 0.37-0.82 μg/mℓ or ~370-820 mg/m^3) did not affect the percent uptake (85.0-94.0%) of toluene in anesthetized dogs exposed on a respirometer. Experiments also indicated that this high level of toluene absorption occurred equally in the upper and lower respiratory tract (Egle and Gochberg, 1976). In mice exposed to 10 μℓ toluene (methyl-^{14}C-radiolabeled) evaporated in 30 mℓ, air retention was ~60% following a 10-minute exposure (Bergman, 1979). The calculated concentration of toluene in the chamber was ~76,660 ppm; however, the maximum level in the chamber would be governed by the saturation of the air, which at 30°C is ~48,900 ppm.

In humans, toluene has been detected in the arterial blood within 10 seconds after initiation inhalation exposures (Astrand et al., 1972; Astrand, 1975). In subjects exposed at 100 or 200 ppm toluene vapor (377 or 754 mg/m^3), toluene concentrations in arterial blood increased rapidly for 10 minutes, began to level off and reached an apparent steady state within 30 minutes. Arterial blood and alveolar concentrations of toluene were dependent on exposure level. At both exposure levels (100 and 200 ppm), however, arterial blood concentration (in mg/kg) and alveolar air concentrations (μg/ℓ) were ~270 and 18% of the inspired air, respectively. These data are consistent with the reported ratio of arterial blood to alveolar air concentration for toluene of ~15 in in vitro tests (Sherwood, 1976; Sato and Nakajima, 1979a). Pulmonary absorption of toluene after exposures of 100-130 ppm for 4 hours resulted in a 40-60% retention and uptake (Nomiyama and Nomiyama, 1974a). Veulemans and Masschelein (1978) reported that in normal healthy males steady-state lung clearance concentrations were reached within 9-13 minutes after exposure to 50 ppm toluene (185 mg/m³) was started.

Several factors have been shown to affect the absorption of toluene from the respiratory tract. Exercise greatly increases the absorption of toluene, probably with increased pulmonary rate and ventilatory volume. Astrand et al. (1972) and Astrand (1975) reported that 30-minute exposures at 100 or 200 ppm (375 or 750 mg/m³) toluene during work at 50 or 150 W caused arterial blood and alveolar air toluene concentrations to be elevated to 620% and 33% or 725% and 47%, respectively. As previously noted, arterial blood and alveolar air concentrations at rest were 270 and 18%, respectively, of the inspired toluene concentration. To rule out the effect of increased heart rate and pulmonary circulation, Astrand et al. (1972) administered 4% CO_2 and toluene (100 ppm) to subjects, which produced increases only in pulmonary ventilation. Arterial and alveolar toluene concentrations increased similarly to those reported during exercise. Carlsson (1982) reported that among 12 subjects exposed at 306 mg/m³, exercise decreased the percent uptake of toluene from ~50-55% at rest to 29% at 150 W exercise. Total uptake (mg of toluene), however, increased 3-fold from rest to 150 W exercise, indicative of increased pulmonary ventilation.

The amount of adipose tissue in the body may affect the absorption of toluene. Carlsson and Lindqvist (1977) suggested that obese men absorbed more toluene, but their data suggested that obese men also inspired more toluene than thin men during a comparative exposure. Veulemans and Masschelein (1978) and Astrand et al. (1972), however, reported no correlation between adipose content and toluene absorption.

Toluene may also be absorbed through the skin, passing slowly into the bloodstream after penetration of epidermal layers. In guinea pigs, in which liquid toluene was applied directly to the clipped skin (a 3.1 cm^2 area on the back) and sealed under glass, the blood-toluene concentration rose to ~1.3 μg/mℓ, then decreased to a steady-state level of ~0.5 μg/mℓ by 6 hours (Jakobson et al., 1982). *In vitro*, toluene was found to penetrate excised rat skin at rates of 0.78 μg/min/cm^2 (Tsuruta, 1982). In this study, 1 mℓ of toluene liquid was applied to a 2.55 cm^2 area of skin in a glass diffusion cell. Approximately 1.55 hours were reported as the lag time of penetration. After 5 hours of exposure, 431±22 μg toluene had penetrated the skin (Tsuruta, 1982).

In humans, dermal absorption was related directly to the concentration of toluene when volunteers immersed both hands in aqueous toluene (180-600 mg/ℓ). Based on the surface area of the hands, the absorption rate was 160-600 μg toluene/cm^2/hour (Dutkiewicz and Tyras, 1968a,b). The absorption rate, following the application of pure toluene to the forearm of subjects, was 14-23 mg/cm^2/hour. Based on these data, Dutkiewicz and Tyras (1968a,b) calculated that immersing both hands in pure toluene for 1 hour would be equivalent to an 8-hour inhalation exposure at 100 mg/m^3. The maximum venous blood-toluene concentration in subjects who immersed one hand in pure toluene for 30 minutes was, however, only 26% of that measured in subjects inhaling toluene at 100 ppm (377 mg/m^3) for 30 minutes (Sato and Nakajima, 1978). Riihimaki and Pfaffli (1978) estimated that dermal absorption in clothed volunteers exposed to ambient air concentrations of 600 ppm (2250 mg/m^3) for ~3 hours was ~1% of the total absorbed dose. The remaining 99% of the total absorbed dose was absorbed by the lungs of the subjects.

Distribution

Toluene, because of its lipophilic nature and low water solubility, would be expected to exist within the body in association with lipid components or adipose tissue. Radiolabeled toluene (4-^{3}H) was administered to male rats by oral intubation (100 μℓ toluene in 400 μℓ peanut oil). Labeled toluene and its metabolites were detected in all tissues examined (white and brown adipose, bone marrow, brain, liver, kidney, muscle, stomach, spleen, adrenal, thyroid, lung, heart and blood) (Pyykko et al., 1977). Tissue levels of radioactivity in liver, lung, heart, thyroid, adrenals and blood peaked 2 hours after administration of the toluene. Three hours after administration of the radiolabeled-toluene and its metabolites, maximum tissue concentrations were recorded in all remaining tissues except white adipose tissue. Distribution of the radioactivity to white adipose tissue occurred more slowly, reaching a maximum after 5 hours. Maximum values may have actually been reached sooner if a different, aqueous vehicle had been used.

Tissue distribution of toluene following inhalation exposure appears very similar to that discussed following oral administration, the one apparent difference being that absorption and, therefore, distribution throughout the body is more rapid by inhalation exposure. Tissue levels in liver, brain and blood of mice exposed for 3 hours to 15 mg/ℓ (15,000 mg/m^3)

toluene rose throughout the exposure period, reaching maximum levels of 625, 420 and 200 mg/kg tissue, respectively (Peterson and Bruckner, 1978; Bruckner and Peterson, 1981a). Similarly, mouse liver, brain and blood tissue levels rose throughout a 10-minute exposure at 40 mg/ℓ (40,000 mg/m³) toluene to reach maximum concentrations of ~230, 140 and 70 mg/kg tissue, respectively. Liver, brain and blood levels of toluene reached 600-700, 400-480 and 220-240 mg/kg, respectively, in mice exposed at 40 mg/ℓ (40,000 mg/m³) intermittently (5 minutes exposed, 10 minutes unexposed or 10 minutes exposed, 20 minutes unexposed) for 3 hours (Peterson and Bruckner, 1978).

Bergman (1979) conducted three-step, low temperature autoradiography analyses on mice exposed to saturated toluene vapor (~48,900 ppm or 184.4 g/m³) (see Absorption Section) to determine the distribution of toluene, its metabolites and covalently bound intermediates. Nonvolatile, water soluble radiolabeled components were considered metabolites and volatile radiolabeled components represented unreacted toluene. Substances not removed by heating or water-soluble or lipid-soluble extraction were presumed to be covalently bound. Nonvolatile metabolites were detected in the liver, kidney and blood soon after exposure. Unmetabolized toluene was found to predominate in adipose tissue and nervous tissue. Apparently no covalently bound metabolic intermediates of toluene were detected.

In adult male rats exposed for 10 minutes to 130 μℓ toluene (4-^{3}H-radiolabeled)/6.5 ℓ air in a respiration chamber (17,340 mg/m³), tissue distribution was very rapid (Pyykko et al., 1977). Maximum tissue concentrations were reached within 15-30 minutes after exposure was terminated in all tissues. The tissues analyzed were the same as those previously noted for oral administration studies conducted by Pyykko et al. (1977) except white adipose tissue. In white adipose tissue, maximum radioactivity was reached 1 hour after exposure ended. Benignus et al. (1981) reported the maximum blood and brain concentrations of toluene in rats to be ~11.0 and 21.0 ppm (mg/kg) after 4 hours of exposure to 566 ppm (2130 mg/m³) toluene in air. Distribution was rapid, reaching near maximal tissue levels within 1 hour of exposure.

Bioaccumulation and Retention

Toluene and its metabolites are removed from tissues relatively quickly after exposure is terminated (Pyykko et al., 1977). In rabbits, the tissue-blood partition coefficients for red bone marrow and peritoneal adipose tissue were determined to be 35.43 and 113.16, respectively (Sato et al., 1974). Toluene will, therefore, accumulate in these tissues if exposure is prolonged. Urinary hippuric acid (used as a measure of toluene present in the body) had returned to normal within 24 hours in rabbits exposed at 2000-3000 ppm toluene for 1 hour (Takahashi, 1982). Inhalation of toluene at 3000-7000 ppm required 48 hours for urinary hippuric acid levels to recover. A 30- to 40-minute exposure at 6000-10,000 ppm toluene required at least 36 hours for urinary hippuric acid levels to recover in rabbits (Takahashi, 1982). Studies by Carlsson and Lindqvist (1977) and Pyykko et al. (1977) indicated that the concentration of radioactivity (toluene or metabolites) was highest in white adipose tissue due to the poor blood perfusion. The accumulation of radiolabeled toluene in these tissues was delayed slightly compared with other tissues, but the loss of radioactivity from adipose tissue and bone marrow appeared to occur more slowly than from other tissues (Pyykko et al., 1977). Bergman (1979), however, reported that the only radioactivity detectable 8 hours after a 10-minute exposure (saturated toluene vapor) in mice was found in the kidney and within intestinal contents.

In experiments with dogs exposed to 400 ppm (1500 mg/m³) toluene, 7 hours/day for 5 days, neither the total amount of excreted hippuric acid nor the venous blood toluene level increased over the exposure period (von Oettingen et al., 1942a). Bioaccumulation over the 5-day period was, therefore, not exhibited.

In human volunteers exposed experimentally to ~300 mg/m³ toluene, analysis of tissue biopsies of subcutaneous body fat indicated that ~0.7 and 9.9 mg toluene/kg, respectively, were concentrated in this tissue following toluene exposure at rest and after 50 W exercise for 2 hours (Carlsson and Lindqvist, 1982). Although there was a decrease in toluene concentration in adipose tissue from obese individuals, the half-life of toluene in these individuals was increased. The half-life of toluene in adipose tissue of lean subjects was ~12 hours, while that in an obese subject was calculated to be ~65 hours. The obese individual had >25% of body weight as adipose tissue.

Following occupational exposure to toluene, the potential for accumulation appears to be low. Ovrum et al. (1978) studied eight printers exposed to toluene at levels of 35-353 ppm (132-1330 mg/m³) over a 5-day work week. Blood samples taken daily before work showed no cumulative effect of toluene. Similarly, Lehnert et al. (1978) observed that urinary hippuric acid levels from 3155 samples from workers exposed to toluene and other industrial solvents could not be correlated to different days of the work week except that samples taken on Monday were significantly higher than other days. Elevated hippuric acid on Monday was regarded as a result of weekend eating habits (some foods contain hippuric acid precursors).

Metabolism

The first step in the metabolism of toluene in animals is the hydroxylation of the side-chain to benzyl alcohol. Hepatic MFOs are primarily responsible for this metabolism (Daly et al., 1968). By analogy with p-nitrotoluene, Ikeda and Ohtsuji (1971) suggested that benzyl alcohol is oxidized further to benzaldehyde by alcohol dehydrogenase. Benzaldehyde is oxidized further by aldehyde dehydrogenase to benzoic acid. Benzoic acid is conjugated with glycine to form hippuric acid or conjugated with glucuronic acid to form benzoyl glucuronide (Figure III-1). Both conjugated metabolites are major urinary excretion products. In rabbits intubated with toluene (300 mg/kg bw), ~74% of the administered dose was excreted as hippuric acid (El Masry et al., 1956).

A minor metabolic pathway for toluene is by ring-hydroxylation. *In vitro*, rat or rabbit liver microsomal preparations have metabolized toluene to minute amounts of o- and p-cresol (Daly et al., 1968; Kaubisch et al., 1972). In male rats treated orally with 100 mg toluene/kg bw, the range for urinary excretion of o-cresol and p-cresol was 0.04-0.11% and 0.4-1.0% of the dose, respectively (Bakke and Scheline, 1970). The cresols are conjugated to sulfates for urinary excretion.

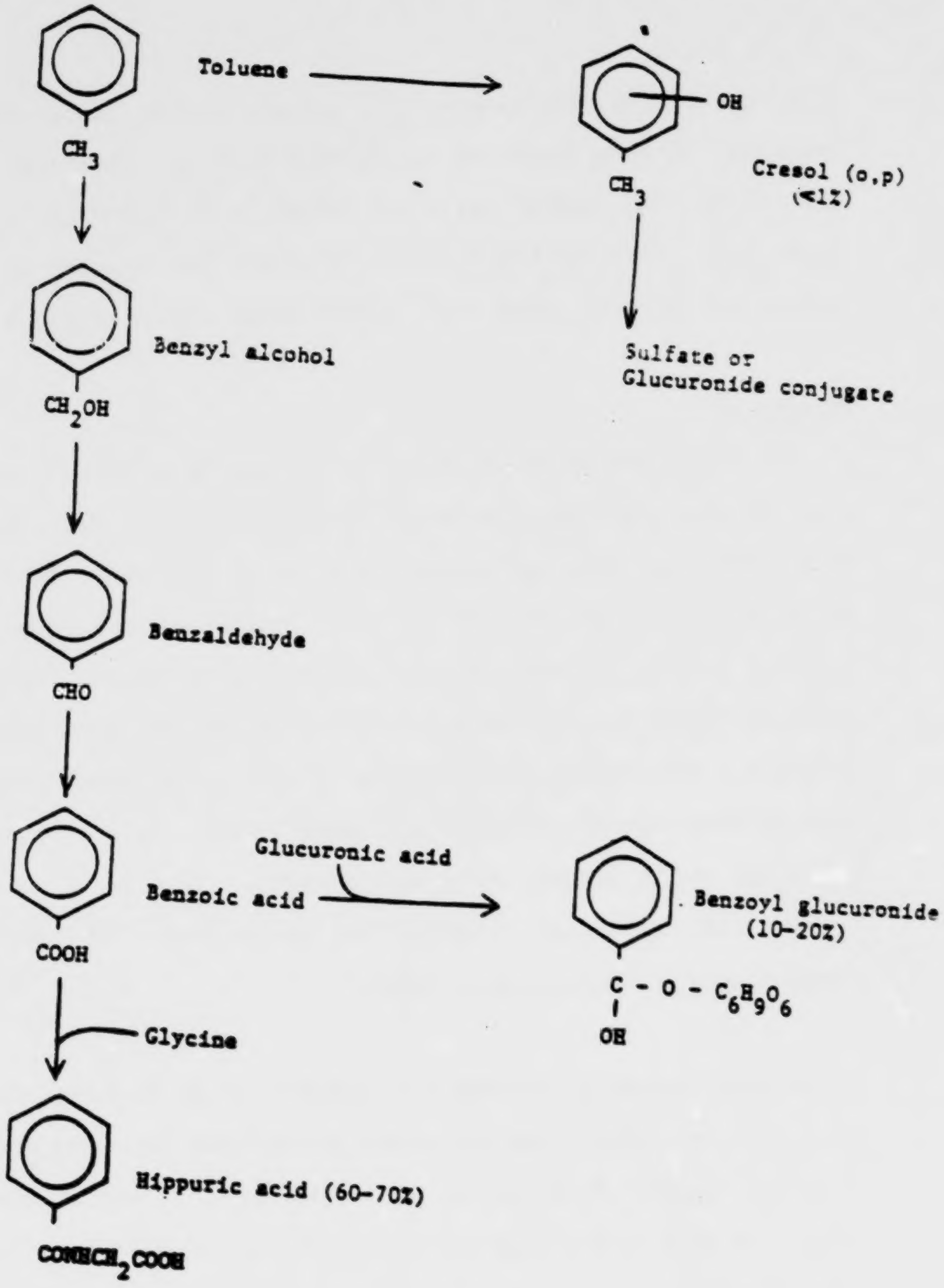

Toluene
OH
CH_3
Cresol (o,p)
(<1%)
CH_3
Sulfate or
Glucuronide conjugate
Benzyl alcohol
CH_2OH
Benzaldehyde
CHO
Glucuronic acid
Benzoic acid
COOH
Benzoyl glucuronide
(10-20%)
$C - O - C_6H_9O_6$
OH
Glycine
Hippuric acid (60-70%)
$CONHCH_2COOH$

FIGURE III-1

Metabolism of Toluene in Humans and Animals

Source: Adapted from El Masry et al., 1956

Another minor metabolite identified in male rats injected i.p. with 370 mg toluene/kg bw is a mercapturic conjugate (Van Doorn et al., 1980). The presumed metabolite, benzylmercapturic acid, was detected as 0.4-0.7% of the administered dose (Van Doorn et al., 1981). The mercapturic conjugate would require glutathione in its formation, and slight decreases in liver glutathione levels were concomitant with toluene exposure (Van Doorn et al., 1980, 1981).

Several studies have indicated that ~60-70% of the absorbed toluene is excreted in the urine as hippuric acid (Ogata et al., 1970; Veulemans and Masschelein, 1979). The metabolism of toluene to hippuric acid is rapid, since elevated urinary hippuric acid is detected within 30 minutes of inhalation exposure (Ogata et al., 1970; Nomiyama and Nomiyama, 1978). Amsel and Levy (1969) reported that ~190 μmol hippuric acid/minute was formed from benzoic acid (2-5 g orally) by three male volunteers. The limiting factor for this maximum rate was the availability of glycine. In fact, glycine treatment in dogs was shown to enhance the excretion of hippuric acid following toluene inhalation (von Oettingen, 1942a). Corroboratively, Riihimaki (1979) reported a constant maximal rate of 180-198 μmol/minute for urinary excretion of benzoic acid as hippuric acid in humans. Assuming pulmonary absorption of 60%, Riihimaki (1979) estimated that the capacity for benzoic acid conjugation and excretion in humans would be saturated at 780 ppm (2940 mg/m³) toluene during light work (10 μ/minute tidal volume) or 270 ppm (1020 mg/m³) during heavy work (30 μ/minute tidal volume). After this capacity is saturated, excretion of metabolites appear to be delayed and accumulation may occur.

Benzoyl glucuronide may account for 10-20% of the absorbed dose of toluene in humans (Srbova and Teisinger, 1953). Urinary o-cresol has been detected in workers exposed to toluene at 7-112 ppm (Angerer, 1979; Pfaffli et al., 1979; Hansen and Dossing, 1982). o-Cresol excretion was proportionate to toluene exposure but accounted for only ~0.05% of the absorbed toluene. Urinary p-cresol may have been elevated in toluene-exposed workers but unexposed workers also had low levels of urinary p-cresol. m-Cresol has been reported as a urinary metabolite in humans exposed to 280 ppm (1060 mg/m^3) toluene, but has not been reported in any other human or animal metabolism studies (Woiwode et al., 1979).

Excretion

Toluene excretion in animals and humans is dose-dependent and occurs by three major means. Urinary hippuric acid accounts for up to 75% of absorbed toluene. Elimination of unchanged toluene vapor from the lungs and possibly from the skin (Sato and Nakajima, 1978) accounts for 10-20% of absorbed toluene. The glucuronide conjugate of benzoic acid excreted in the urine may represent 10-20% of the absorbed toluene.

In rabbits administered 350 mg toluene/kg bw by oral intubation, 18% of the dose was expired as toluene vapor within 14.5 hours of treatment. Only 1% more was expired from 14.5-35 hours postexposure (Smith et al., 1954). Data from the same laboratory (El Masry et al., 1956) indicate that rabbits intubated with 274 mg toluene/kg bw excreted urinary hippuric acid that accounted for 74% of the dose. Excretion was considered complete within 24 hours of the oral dosing.

Excretion of toluene and its metabolites is rapid following oral and inhalation exposure. Mice exposed to saturated toluene vapor (184,350 mg/m^3) for 10 minutes excreted ~10 and 68% of the absorbed dose as expired toluene and urinary metabolites, respectively, within 8 hours (Bergman, 1979). Pyykko et al. (1977) noted that by 24 hours after inhalation exposure (4600 ppm 4-^3H toluene for 10 minutes) in rats, <1% of the radioactivity remained in most tissues, while ~5% remained in white adipose tissue. Urinary hippuric acid reached maximum levels within 1.5 hours after exposure to 350 ppm (1320 mg/m^3) for 100 minutes or 4500 ppm (17,000 mg/m^3) for 10 minutes (Nomiyama and Nomiyama, 1978). Hippuric acid excretion had returned to normal by 7 and 3 hours, respectively, after exposure to 350 ppm (100 minutes) and 4500 ppm (10 minutes).

The excretion of toluene in the expired air of 5 men and 5 women was reported to be 17.6 and 9.4%, respectively, of the absorbed dose (Nomiyama and Nomiyama, 1974b). Veulemans and Masschelein (1978) determined that only ~4% of the absorbed dose was expired as toluene vapor in male subjects exposed on various discontinuous dose schedules (longest continuous exposure was 50 minutes). Several factors may contribute to differences in the percentages of toluene excreted through the lungs. Veulemans and Masschelein (1978) noted that working subjects (6 males) exhaled less toluene (% of absorbed dose) than did the subjects when resting. The amount of body fat also may affect the extent of toluene expiration since a negative correlation ($r^2 = 0.2134$) was observed (Veulemans and Masschelein, 1978). This suggests that an obese person would expire less toluene than a thin person.

The excretion of hippuric acid, which may account for 60-70% of absorbed toluene, is quite rapid and usually complete within 24 hours after exposure (Veulemans and Masschelein, 1979). The excretion rate of hippuric acid increased during the first 2 hours but appeared to reach a plateau (at ~0.9, 1.4 and 2.0 mg urinary hippuric acid/minute) by the third hour in human subjects exposed to 50, 100 or 150 ppm toluene (189, 377 or 566 mg/m³, respectively) at rest (Veulemans and Masschelein, 1979). Exercise increased the rate of hippuric acid excretion. The half-life for urinary hippuric acid was determined to be 117 minutes for men and 74 minutes for women (Nomiyama and Nomiyama, 1978) or between 2.0 and 2.3 hours (Veulemans and Masschelein, 1979).

In experiments simulating exposure in the workplace (3 hours of exposure followed by 1 unexposed hour and 4 subsequent exposure hours), 4-5 male volunteers were exposed to 200 ppm (754 mg/m³) toluene and monitored for excretion of hippuric acid (Ogata et al., 1970). Hippuric acid excretion rose continuously during the first 3 hours of exposure, reaching a rate of ~3 mg/minute (corresponding urinary concentration of 6 mg/mℓ). During the unexposed period and ~1 hour into the second exposure period, hippuric acid excretion rate and urinary concentration remained relatively constant. After ~1 hour into the second (4-hour) exposure period, excretion rate and urinary concentration increased to 4.5-5 mg/minute and 10 mg/mℓ hippuric acid, respectively. The excretion rate did not begin to decline until ~2 hours after the end of toluene exposure. Within 18 hours after exposure was terminated, urinary excretion of hippuric acid was near baseline levels (Ogata et al., 1970).

The use of urinary hippuric acid levels to estimate toluene exposure among workers essentially has been ruled out as a viable screening measure. Imamura and Ikeda (1973) noted that the level of urinary hippuric acid in workers exposed to ≤100 ppm toluene (the TLV) is not increased greatly above normal hippuric acid concentrations. Several investigators have reported considerable variation in urinary hippuric acid between unexposed humans (normal levels) and workers exposed to low levels of toluene (Ikeda and Ohtsuji, 1969; Imamura and Ikeda, 1973; Wilczok and Bieniek, 1978). Physiological and anatomical differences, such as body fat, cardiopulmonary parameters, body weight and sex, affect hippuric acid excretion. Exercise and exposure conditions influence toluene uptake and, therefore, alter hippuric acid excretion.

The body burden of toluene, determined by alveolar air, arterial blood and venous blood concentrations in humans exposed by inhalation, declines quite rapidly after exposure is terminated. Although two- and three-stage models of toluene excretion have been proposed (Nomiyama and Nomiyama, 1974b; Veulemans and Masschelein, 1978; Sato and Nakajima, 1979b), the half-life of toluene decay of the slowest phase has been reported as 114, 124 and 221 minutes. In any case, the clearance of toluene from the body is rapid.

Summary

Toluene appears to be absorbed rapidly by oral, inhalation and dermal routes. Absorption from the GI tract seems to be complete (>99%), while pulmonary absorption is ~85-90% during brief exposures but may be nearer to 50% during extended exposures (>1 hour). Toluene is distributed throughout the body with greatest accumulation in lipid tissues (adipose, bone marrow).

Long-term bioaccumulation of toluene or its metabolites does not seem likely. Metabolism and urinary excretion of toluene accounts for 80-90% of its elimination, while the remaining absorbed toluene is expired as the parent compound. The major urinary metabolites are hippuric acid (60-75% of absorbed dose) and benzoyl glucuronide (up to 20%), while several cresols (ring-hydroxylated metabolites) have been reported. Excretion of toluene is rapid but its rate may depend somewhat on dose and the supply of glycine. Excretion of toluene metabolites is delayed somewhat after oral exposure when compared with inhalation. By either route, excretion would be expected to be complete within ~24 hours of exposure. Glycine supply, needed for conjugation in hippuric acid formation, may be a limiting factor in the rate of toluene excretion. Riihimaki (1979) suggested that toluene at 780 ppm (2940 mg/m³) during light work or 270 ppm (1020 mg/m³) during heavy work would saturate the capacity for glycine conjugation in humans. Subsequently, toluene may accumulate or be metabolized by alternate pathways.

IV. HUMAN EXPOSURE

Detailed information concerning the occurrence of and exposure to toluene in the environment is presented in the draft document entitled "Occurrence of Synthetic Organic Chemicals in Drinking Water, Food, and Air" (SAIC, 1987). This chapter summarizes the pertinent information presented in that document to assess the exposure from drinking water, food and air.

Humans may be exposed to toluene from a variety of sources. This analysis is limited to exposures in drinking water, food and ambient air. Some individuals may also receive significant exposures to toluene from consumer products and from their occupational environment. Individual exposure to toluene will vary and will be affected by age, weight and lifestyle.

The Exposure Estimates Section of this chapter presents available information on the range of human exposure to and intake of toluene from drinking water, food and ambient air. It is not possible to provide an estimate of the number of individuals experiencing specific exposures from these three sources. However, this section provides some insight into the relative contributions of the three sources.

Use/Environmental Fate

Toluene is produced primarily from three sources: petroleum refining processes, as a by-product of styrene production, and as a by-product of coke-oven operations. In 1984, U.S. production of toluene was estimated to be 5.3 billion pounds with the largest source being petroleum refining processes (USITC, 1985; U.S. EPA, 1983). Toluene is released into the environment during the many phases of its production, use and disposal (U.S. EPA, 1983, 1986). The largest releases occur as a result of its use in gasoline and as a solvent (U.S. EPA, 1983). While the majority of releases are to air, significant amounts are released to surface and groundwater.

Once released into the environment, toluene readily volatilizes from surface soils and surface waters (Lyman et al., 1982). The volatilization half-life of toluene in surface waters and some shallow soils ranges from <1 minute to several days depending on the environmental conditions. Toluene is not expected to undergo significant rates of removal from soil or water by processes of hydrolysis, direct photolysis or oxidation by peroxy radicals. Oxidation of toluene by hydroxyl free radicals (OH.) appears to be significant from both natural waters and the atmosphere (Lyman et al., 1982; U.S. EPA, 1984; Mabey et al., 1981; Jefcoate et al., 1969). Biodegradation may also be an important process for the removal of toluene from surface water, and soil (Mabey et al., 1981; U.S. EPA, 1984; Callahan et al., 1979). In soil, toluene is expected to be somewhat immobile and moderately resistant to leaching.

Based on its physical and chemical properties, toluene is not expected to bioaccumulate in aquatic life (U.S. EPA, 1987).

Occurrence

Drinking Water. The occurrence of toluene in groundwater and surface water supplies has been evaluated on both the national and regional levels. Toluene has been analysed for in four national surveys and in a number of regional surveys [see the occurrence document U.S. EPA (1987) for details].

The surveys found that toluene was a relatively rare contaminant in drinking water. The levels of toluene that were reported generally did not exceed a 10 µg/ℓ. State and regional data generally found similar concentrations of toluene (U.S. EPA, 1987).

Diet. The available data on the occurrence of toluene in food are very limited. Several studies have been identified that address the subject, but the information presented as results of those studies was not extensive enough to estimate daily intake levels. In general, those studies indicated that toluene, where it did occur, did not exceed a few 1000 µg/kg. The U.S. EPA believes that toluene is not a major contaminant of food. Toluene has only a limited occurrence in surface waters and does not bioaccumulate in animals. Because of toluene's volatility, levels in food are not expected to be high. The limited available data support this conclusion.

Air. Toluene is a common low level contaminant in ambient air. Brodzinsky and Singh (1982) compiled data on the occurrence of toluene in ambient air, which they used to develop the following median environmental concentrations: rural/remote areas, 2.5 µg/m^3; urban/suburban areas, 41 µg/m^3; and source-dominated areas, 17 µg/m^3.

The U.S. EPA has performed a study of volatile contaminants in indoor air (U.S. EPA, 1986). While this study did not report the levels of toluene in indoor air, it found that similar chemicals (benzene, xylenes, ethyl benzene, and styrene) commonly occur in houses at levels much higher than ambient air. The survey found that some individuals received intakes for these compounds of >10,000 mg/day. Toluene commonly co-occures in petroleum and consumer products with these other compounds and has similar chemical and physical properties. The U.S. EPA suspects that toluene may have similar levels of indoor air exposure.

Exposure Estimates

The estimates of exposure to toluene from drinking water, food and ambient air in Table IV-1 were derived using the assumptions that an average adult intakes 2 ℓ of water/day, and 20 m^3 of air/day. The estimates of exposure are presented in ranges to reflect the uncertainty of the limited data available. The levels of toluene in air are taken from studies of toluene in ambient air. Indoor levels of toluene may be higher. No estimate of intakes of toluene from diet were made due to the lack of data.

The information currently available on the occurrence of toluene in the environment and the potential for exposure is insufficient to determine the distribution of intake on a national scale from any of the three sources. The data above suggests that for most individuals, the daily intake of toluene should be relatively low. However, this assessment may underestimate intake from indoor air. Data for similar compounds suggest that exposure to toluene in indoor air is probably much greater then the above levels suggest. The intake of toluene from food, while not estimated, is expected to be small. Actual data on the occurrence of toluene, especially in indoor air and food, are needed before more accurate estimates of toluene intake can be made.

TABLE IV-1

Exposure Estimates for Toluene

Source	Range of Reported Exposure Levels (low-high)	Estimated Daily Intake
Drinking Water	0-10 $\mu g/\ell$	0-20 μg/day
Diet	Data not sufficient	--
Air	2.5-41 $\mu g/m^3$*	50-800 μg/day

*Does not reflect indoor air exposure.

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V. HEALTH EFFECTS IN ANIMALS

General Toxicity

Animal studies of toluene toxicity have been conducted predominantly to simulate and evaluate risks of exposure in humans. Acute studies are reflective of high level exposures in humans, such as abuse of toluene (i.e., glue sniffing) or solvent spills. Chronic tests are designed to provide information relevant to industrial-type exposure. Because of the nature of human exposure, inhalation is the most common route of administration in animal studies.

Acute Toxicity. The limited number of studies on the acute oral toxicity of toluene focus predominantly on lethal effects. Wolf et al. (1956) treated 26 male and female Wistar-derived rats with a single oral dose of undiluted toluene (94.4% purity; impurities not specified) by stomach intubation. Based on mortality data collected over a 2-week observation period, the LD_{50} for young adult rats (175-250 g bw) was 7.0 g/kg bw. Following a single dose by gastric intubation to 5 male Carworth-Wistar rats, the 14-day LD_{50} was determined to be 7.3 g/kg bw (Smyth et al., 1969a). The LD_{50} values for young adult (80-160 g bw) and older adult (300-470 g bw) rats were 6.4 and 7.4 mℓ/kg bw (5.5 and 6.4 g/kg bw), respectively (Kimura et al., 1971). These studies were conducted using six male Sprague-Dawley rats/group intubated with undiluted analytical grade toluene and observed for mortality for 1 week postexposure.

Withey and Hall (1975) studied the acute lethal effects of toluene administered by gastric intubation in male Sprague-Dawley Cobb rats. Young adult rats (150-200 g bw) were fasted overnight just before dosing and observed three times daily for at least 7 days postexposure. LD_{10}, LD_{50} and LD_{90} values were estimated by probit analysis of the dose-response data from five dose levels. In a pilot study, three groups of 10 rats were dosed at 4.00, 6.76 and 11.00 g/kg and mortality was 1/10, 9/10 and 10/10, respectively. In the main experiment, five groups of 20 animals each were exposed at 4.00, 4.56, 5.20, 5.93 and 6.76 g/kg bw. Mortality among these groups was 1/20, 0/20, 9/20, 13/20 and 17/20, respectively, resulting in LD_{10}, LD_{50} and LD_{90} values of 4.51, 5.58 and 6.91, respectively (Withey and Hall, 1975). Toxic effects or pathologic findings of the toluene-treated animals were not reported for this study.

The oral LD_{50} of toluene in rats is somewhat dependent on the age of test animals. In adult rats, the LD_{50} following a single oral exposure was between 5.5 and 7.53 g/kg (Wolf et al., 1956; Smyth et al., 1969a; Kimura et al., 1971). In younger adults, the value was slightly lower, ranging from 5.3-5.9 g toluene/kg (Kimura et al., 1971; Withey and Hall, 1975). Using 6-12 rats/group and the procedures reported previously for this study, Kimura et al. (1971) determined that the LD_{50} in 14-day-old rats was 2.6 g/kg. Newborn rats were very sensitive so that <1 g/kg bw was lethal. The "first observable signs of drug action," presumably a CNS effect, by toluene was reported at 2.0 mℓ/kg (1.7 g/kg bw) for young adult rats (Kimura et al., 1971). Based on these data, a maximal limit for a single oral dose was suggested by Kimura et al. (1971) as 2.0 μℓ/kg bw (1.7 mg/kg bw).

A summary of the acute toxic effects of toluene by various routes of administration in animals is presented in Table V-1.

TABLE V-1

Acute Effects of Toluene

Route	Species (no. sex)	Dose	Effect	Reference
Oral (gavage)	Carworth-Wistar rats (5 males)	7.53 g/kg (range, 6.73-8.43 g/kg)	LD_{50}	Smyth et al., 1969a
Oral (gavage)	Wistar rats (10 females)	7.0 g/kg	LD_{50}	Wolf et al., 1956
Oral (gavage)	Sprague-Dawley rats (20 males)	5.58 g/kg (range, 5.3-5.9 g/kg)	LD_{50}	Withey and Hall, 1975
Oral (gavage)	Sprague-Dawley rats 14 days old (6-12 males and females)	3.0 ml/kg (2.6 g/kg)	LD_{50}	Kimura et al., 1971
	young adults (6-12 males and females)	6.4 ml/kg (5.5 g/kg)	LD_{50}	
	older adults (6-12 males and females)	7.4 ml/kg (6.4 g/kg)	LD_{50}	
Inhalation	rats (no. and sex not reported)	1600 ppm for 18-20 hours	Initial effect of instability and incoordination, conjunctivitis, lacrimation and sniffles; then narcosis	Batchelor, 1927
Inhalation	rats (no. and sex not reported)	1600 ppm for 18-20 hours/day x 3 days	Mild twitching; drop in body temperature; death. Histology: severe cloudy swelling of kidneys, no effect on liver, heart or testes	Batchelor, 1927
Inhalation	rats (no. and sex not reported)	1250 ppm for 18-20 hours	Slight instability and incoordination; mucous membrane irritation	Batchelor, 1927
Inhalation	rats (no. and sex not reported)	620 ppm, 1100 ppm for 18-20 hours	No-effect-level on symptoms; hyperplasia of bone marrow due to benzene contaminant	Batchelor, 1927
Inhalation	rats (10, sex not reported)	24,400 ppm for 1.5 hours	60% mortality	Cameron et al., 1938
Inhalation	rats (10, sex not reported)	12,200 ppm for 6.5 hours	50% mortality	Cameron et al., 1938
Inhalation	rats (6, sex not reported)	4000 ppm for 4 hours	1/6 dead	Smyth et al., 1969b
Inhalation	Sprague-Dawley rats (12 males)	4618 ppm for 6 hours (99.5% purity)	LC_{50}	Bonnet et al., 1982

TABLE V-1 (cont.)

Route	Species (no. sex)	Dose	Effect	Reference
Inhalation	mice (10, sex not reported)	24,400 ppm for 1.5 hours	10% mortality	Cameron et al., 1938
Inhalation	mice (10, sex not reported)	12,200 ppm for 6.5 hours	100% mortality	Cameron et al., 1938
Inhalation	Swiss mice (16-20, sex not reported)	5320 ppm for 7 hours (<0.01% benzene present)	LC_{50}	Svirbely et al., 1943
Inhalation	mice (no. and sex not reported)	6949 ppm for 6 hours (99.5% purity)	LC_{50}	Bonnet et al., 1982
Inhalation	guinea pigs (3, sex not reported)	4000 ppm for 4 hours	2/3 dead within a few days	Smyth and Smyth, 1928
Inhalation	rabbits (no. and sex not reported)	5500 ppm	Lethal within 40 minutes	Carpenter et al., 1944
Inhalation	dogs (3, sex not reported)	850 ppm for 1 hour (0.01% benzene)	Increased respiratory rate, decreased respiratory volume	von Oettingen et al., 1942a
Dermal (single application)	New Zealand rabbits (4 males)	14.1 mℓ/kg	LD_{50}	Smyth et al., 1969a
Dermal, abdomen	New Zealand rabbits (4 males)	uncovered application	Slight irritation	Smyth et al., 1969a
Dermal	rabbits (no. and sex not reported)	10-20 applications of undiluted toluene to rabbit ear and bandaged to shaved abdomen	Perceptible erythema, thin layer of devitalized tissue that exfoliated. No effect on gross appearance, behavior or weight	Wolf et al., 1956
Dermal	guinea pigs (20, sex not reported)	1 mℓ (0.87 g) for 16 hours	Karyopyknosis, karyolysis, perinuclear edema, spongiosis, junctional separation, cellular infiltration in dermis, no liver and kidney damage	Kronevi et al., 1979
Corneal	rabbits (no. and sex not reported)	0.005 mℓ (0.004 g)	Moderately severe injury	Smyth et al., 1969a
Corneal	rabbits (no. and sex not reported)	0.005 mℓ (0.004 g)	Moderately severe injury	Carpenter and Smyth, 1946
Corneal	rabbits (no. and sex not reported)	2 drops	Perceptible irritation of conjunctival membranes. No corneal injury.	Wolf et al., 1956

TABLE V-1 (cont.)

Route	Species (no. sex)	Dose	Effect	Reference
i.p. injection	rats and mice (no. and sex not reported)	2.0 ml/kg (1.7 g/kg)	Lethal dose	Cameron et al., 1938
i.p. injection	rats (no. and sex not reported)	0.75 ml/kg (0.7 g/kg)	Apathy; unresponsive behavior	Batchelor, 1927
i.p. injection	rats (no. and sex not reported)	1.75-2.0 ml/kg (1.5-1.7 g/kg)	Death from respiratory failure	Batchelor, 1927
i.p. injection	Sprague-Dawley rats (equal no. of males and females, no. not reported)	800 mg/kg at 26°C 530 mg/kg at 8°C 255 mg/kg at 36°C	Approximate lethal dose	Keplinger et al., 1959
i.p. injection	ddN mice (6 males)	1.15 g/kg in olive oil (1.04-1.31 g/kg, graded doses between 0.79 and 1.65 g/kg)	LD_{50}; observed for 24 hours. Cause of death: respiratory failure	Koga and Ohmiya, 1978
i.p. injection	Wistar rats (females, no. not reported)	1.64 g/kg	LD_{50}	Ikeda and Ohtsuji, 1971
s.c. injection	rats and mice (no. and sex not reported)	5-10 ml/kg (4.3-8.7 g/kg)	Lethal dose	Cameron et al., 1938
i.v. injection	rabbits (15, sex not reported)	0.15 ml/kg (0.13 g/kg) 0.20 ml/kg (0.17 g/kg)	13% mortality 100% mortality	Braier, 1973

The LC_{50} for toluene (<0.01% benzene) inhalation for 7 hours in Swiss mice was 5320 ppm (20,000 mg/m³) (Svirbely et al., 1943). The acute toxicity of toluene administered by inhalation was studied in rats and mice (Cameron et al., 1938). Exposure to two dose levels, 12,200 and 24,400 ppm (46,000 and 92,000 mg/m³) toluene was for 6.5 and 1.5 hours, respectively. In rats, mortality was 50 and 60%, respectively, following exposure to the lower (6.5 hours) and higher (1.5 hours) toluene concentrations. Mortality in mice was 100 and 10% following exposure to toluene at 12,200 and 24,400 ppm for 6.5 or 1.5 hours, respectively. Bonnet et al. (1982) reported that the LC_{50} for toluene (99.5% purity), following 6 hours of inhalation, was 4618 ppm (17,400 mg/m³) in Sprague-Dawley rats. Bonnet et al. (1982) reported that the LC_{50} following 6 hours of inhalation was 6949 ppm (26,200 mg/m³) toluene (99.5% purity) in mice. Although a 14-day observation period was allowed after exposure, most deaths occurred during the 6-hour exposure or within 24 hours postexposure. Smyth et al. (1969b) reported that 1 of 6 rats died as a result of exposure to 4000 ppm (15,080 mg/m³) technical grade toluene for 4 hours.

In an early study, Batchelor (1927) reported that a single 18- to 20-hour pulmonary exposure to 1600 ppm (6030 mg/m³) toluene in rats caused a progression of effects ranging from instability and incoordination to narcosis with conjunctivitis, lacrimation and nasal irritation. Exposure to toluene (1600 ppm in air, 18-20 hours/day) for 3 consecutive days caused muscular twitching, decreased body temperature and death. Histopathological examination of animals exposed for 3 days identified severe cloudy swelling of the kidneys but no effect on liver, heart or testes. In rats exposed to 1250 ppm (4710 mg/m³) toluene in air for a single 18- to 20-hour interval, only slight effects on coordination and mucous membrane irritation were evident. At a concentration of 620 or 1100 ppm (2340 or 4150 mg/m³), no effects were attributed to toluene. Bone marrow hyperplasia identified in these rats was suggested as an effect of the benzene contaminant (Batchelor, 1927).

Following toluene exposure to 4000 ppm (15,080 mg/m³) for 4 hours, 2 of 3 guinea pigs died within a few days (Smyth and Smyth, 1928). A concentration of 5500 ppm (20,700 mg/m³) toluene was lethal within 40 minutes in exposed rabbits (Carpenter et al., 1944). Increased respiratory rate and decreased respiratory volume were noted in dogs inhaling toluene (0.01% benzene) at 850 ppm (3200 mg/m³) for only 1 hour (von Oettingen et al., 1942a).

Dermal exposure to toluene in rabbits caused slight irritation with erythema and some desquamation (Wolf et al., 1956; Smyth et al., 1969a). Kronevi et al. (1979) reported nuclear changes in dermal cells and cellular infiltration of the dermis in guinea pigs treated with 1 mℓ (0.87 mg) of toluene under glass for 16 hours. The LD_{50} for dermally applied toluene in rabbits (single application) was 14.1 mℓ/kg (12.2 g/kg) (Smyth et al., 1969a).

Moderately severe corneal injury was evident in rabbits having 0.005 mℓ (0.004 g) of toluene placed in the eyes (Carpenter and Smyth, 1946; Smyth et al., 1969a). Wolf et al. (1956) reported that two drops (~0.05 mℓ) of toluene placed in the eyes of rabbits caused conjunctival irritation, but no apparent corneal injury.

The LD_{50} values for male and female mice were 1.15 and 1.64 g/kg, respectively, when toluene was injected i.p. in olive oil (Koga and Ohmiya, 1978). Respiratory failure was reported as the cause of death in rats and mice injected i.p. (Batchelor, 1927; Koga and Ohmiya, 1978). Cameron et al. (1938) reported that the lethal dose in rats and mice given an i.p. injection was 2.0 mℓ/kg (1.7 g/kg), while the lethal dose following s.c. injection was between 5 and 10 mℓ/kg (4.3-8.7 g/kg). Mortality was 13 and 100%, respectively, in rabbits injected i.v. with 0.15 mℓ toluene/kg (0.13 g/kg) and 0.20 mℓ toluene/kg (0.17 g/kg) (Braier, 1973).

Subchronic and Chronic Toxicity. Only one study is available in which laboratory animals were exposed to toluene by the oral route. In this study (Wolf et al., 1956), three groups of 10 female Wistar-derived rats each were intubated at 118, 354 or 590 mg toluene/kg/day, respectively, in olive oil for 193 days (5 days/week for 138 total doses). A group (n=20) gavaged with only olive oil (2.5 mℓ) on the same dose schedule as toluene-treated rats served as controls. The authors noted that the NOEL was ≥590 mg/kg bw/day since no effects were evident at any dose level. Parameters considered in judging a toxic effect included gross appearance (behavior), growth, hematological tests, BUN, bone marrow counts, final body weight and organ weights, and gross and histopathological examination of liver, kidneys, lungs, heart, spleen, pancreas and adrenals.

There are many subchronic inhalation studies on the effects of toluene in rats, mice, guinea pigs, dogs and monkeys (Table V-2). In rats exposed to 200 or 600 ppm (754 or 2260 mg/m³) toluene for 7 hours/day, 5 days/week over a 6-week period, no significant effects were reported for blood

TABLE V-2

Subchronic Effects of Toluene

Species (no. sex)	Route	Dose	Effect	Reference
Rat/Wistar (10 females/group)	oral	118, 354, 590 mg/kg/day x 138 doses (on 5 days/week)	None; parameters observed: body and organ weights, adrenals, pancreas, femoral bone marrow, lungs, heart, liver, kidney, spleen, testes, bone marrow, BUN, blood counts	Wolf et al., 1956
Rat (NR)	inhalation	200, 600 ppm (754, 2260 mg/m^3) x 7 hours/day x 5 days x 6 weeks	No narcosis; body weight normal; no significant change in WBC, RBC count or hemoglobin; increase in percentage of segmented cells. Histological changes: slight pulmonary irritation; few casts in straight collecting tubules in rats at 600 ppm; no change in liver, spleen, heart and bone marrow	von Oettingen et al., 1942a
Rat (30/dose)	inhalation	2500, 5000 ppm (9425, 18,850 mg/m^3) x 7 hours/day x 5 days/week x 5 weeks	Transient decrease in body weight; increased liver weight, decreased kidney and spleen weight; hyper activity, marked incoordination, recovery after cessation of exposure; mortality in 5000 ppm group 10/25; increased bleeding time; total leucocytes reduced after each exposure; pulmonary lesions occurred earlier than in group exposed to 200 or 600 ppm; casts in renal tubules in all rats within 2 weeks of exposure; reduced staining in hepatocytes, normal spleen, heart and bone marrow histology	von Oettingen, et al., 1942a
Rat (30), guinea pig (30), dog (4), monkey (6)	inhalation	107 ppm continuously for 90 days; or 1085 ppm 8 hours/day, 5 days/week x 6 weeks	No effect on leukocytes, hemoglobin or hematocrit; no effect on liver, kidney, lungs, spleen or heart; no effect on brain or spinal cord of dogs and monkeys	Jenkins et al., 1970
Rats (males)	inhalation	7 consecutive cycles daily, 5 days/week x 8 weeks; each cycle, 10 minutes to 12,000 ppm followed by 20 minutes solvent-free interval	Depression of body weight gain; increased SGOT, LDH levels; no effect on BUN levels; depression of kidney, brain and lung weights. Histology: no effect on brain, lung, liver, heart or kidney, no sign of lipid vacuolation in liver	Bruckner and Peterson, 1981b
CFY rats (no. NR, both sexes)	inhalation	1000 mg/m^3, 6 hours/day x 5 days/week x 1, 3 or 6 months	Bromsulphthalein retention decreased; Cytochrome P-450 increased independent of period of exposure; SGOT and SGPT activity unaffected; decreased body weight and increased liver weight in females	Ungvary et al., 1980

TABLE V-2 (cont.)

Species (no. sex)	Route	Dose	Effect	Reference
CFY rats (no. NR, males)	Inhalation	3500 mg/m³, 8 hours/day x 5 days/week x 1 week, 6 weeks, 6 months	Cytochrome P-450 increased independent of exposure; no effect on SGOT, SGPT, aniline hydroxylase of aminopyrine N-demethylase activity; increased cytochrome b_5 concentrations. Histological effects: dilation of cisternae of rough endoplasmic reticulum of hepatocytes; increase of autophagous bodies, which was dose and time dependent; liver glycogen content decreased; body weight significantly ($p<0.01$) decreased. Relative liver weight increased	Ungvary et al., 1980
CFY rats (no. NR, males)	Inhalation	1500, 3000, 6000 mg/m³ 8 hours/day x 5 days/week x 4 weeks	Cytochrome P-450 increased with dose. Significant ($p<0.01$) increased relative liver weight at higher two doses	Ungvary et al., 1980
Sprague-Dawley rats (both sexes, 15/group)	Inhalation	0, 100, 1500 ppm, 6 hours/day x 5 days/week x 26 weeks	No significant changes in histology or hematology, blood chemistry or urine at any dose	American Petroleum Institute, 1980
Mice/NA2 (male, 6/group)	Inhalation	1, 10, 100, 1000 ppm x 6 hours/day x 20 days	No effect on body weight; 1 and 10 ppm caused increase of RBC count on 10th day; recovery on day 20; 100 ppm, 1000 ppm - decrease of RBC count; all doses - increase (40-70%) of WBC count on day 10; recovery for all doses except 1000 ppm; 10-1000 ppm - decrease of thrombocytes; histology: 100 ppm - slight decrease in density of bone marrow cells and in megakaryocytes and red cell elements; 1000 ppm - slight hypoplasia of red cell elements; slight to moderate disturbance in maturity of neutrophils and thrombocytes, moderate increase of reticulocytes; no change in brain, lung, liver, spleen or kidney.	Horiguchi and Inoue, 1977
Mice/ICR (4-6 males)	Inhalation	7 consecutive cycles daily, 5 days/week x 8 weeks; each cycle, 10 minutes to 12,000 ppm followed by 20 minutes solvent-free interval	Depression of body weight gain; no effect on LDH; decreased BUN levels; SGOT levels increased (not significantly); depression of kidney, brain and lung weights; histology: no effect on brain, lung, liver, heart or kidneys; no sign of lipid vacuolation in liver.	Bruckner and Peterson, 1981b
Mice/ICR (4-6 males)	Inhalation	4000 ppm 99.9% pure toluene for 3 hours/day x 5 days/week x 8 weeks	Depression of body weight gain during first 7 weeks; increased liver-to-body weight ratio after 4 weeks exposure, no effect at 1, 2 or 8 weeks; no increase in kidney, brain and lung weights; SGOT activity increased after 4 weeks of exposure and 2 weeks postexposure, but not after 2 or 8 weeks of exposure; no change in BUN. Histology: no effect on heart, lung, kidney, brain and liver	Bruckner and Peterson, 1981a

TABLE V-2 (cont.)

Species (no. sex)	Route	Dose	Effect	Reference
Guinea pig (NR)	Inhalation	1250 ppm 4 hours/day x 6 days/week (18 exposures)	Prostration, marked liver and renal degeneration, marked pulmonary inflammation	Smyth and Smyth, 1928
		1000 ppm 4 hours/day x 6 days/week (35 exposures)	No symptoms; slight toxic degeneration in liver and kidney	
Dogs (n=2 experimental, 1 control)	Inhalation	2000 ppm 8 hours/day x 6 days/week x 4 months, and then 2660 ppm 8 hours/day, 6 days/week x 2 months	Death on days 179 and 180; slight nasal and ocular irritation; motor incoordination and paralysis of extremities during terminal phase; congestion in lungs, hemorrhagic liver, reduced lymphoid follicles and hemosiderosis in spleen; hyperemic renal glomeruli; albumin in urine	Fabre et al., 1955
Dogs (4 females/group)	Inhalation	200, 400, 600 ppm 3 x 8 hours exposure for 1 week, then 5 x 7 for 1 week and finally 850 ppm for 1 hour	No effect on circulation, spinal pressure; increase of respiratory rate, small increase of minute volume, decrease of respiratory volume. Histology: pulmonary irritation, congestion, edema and hyaline casts in collecting tubules of kidney	von Oettingen et al., 1942b
Rats (NR)	s.c. injection	1 cc/kg (0.87 g/kg) x 21 days	Slight induration at injection site; 5-14% loss of body weight; transient slight drop in RBC and WBC counts; hyperplasia of bone marrow; moderate hyperplasia of malpighian corpuscle in spleen; marked pigmentation of spleen; focal necrosis in liver, slight cloudy swelling in kidney; no effect on heart, testes or lung	Batchelor, 1927
Guinea pig (NR)	s.c. injection	0.25 cc/day (0.22 g/day) x 30-70 days	Local necrosis at injection site; survival period: 30-70 days; polypnea and convulsions during last days of survival; hemorrhagic, hyperemic and sometimes degenerative changes in lungs, kidneys, secondary adrenals, liver and spleen	Sessa, 1948

NR - Not reported; WBC - white blood cell; RBC - red blood cell parameters, body weight gain or histology of the liver, spleen, heart or bone marrow (von Oettingen et al., 1942a). Slight pulmonary irritation was evident in rats of both dose groups. Urinary casts were identified in the collecting tubules of rats treated at 600 ppm toluene. In rats exposed to 2500 or 5000 ppm (9425 or 18,850 mg/m³) toluene for the same schedule (7 hours/day, 5 days/week) but for 5 weeks' duration; toxic effects were more pronounced and occurred earlier in the exposure period (von Oettingen et al., 1942a). Casts within the renal tubules were evident within 2 weeks at both dose levels and pulmonary lesions were more extensive. In addition, the body weight of exposed rats decreased; exposed rats also showed dose-related incoordination and narcosis during each daily exposure period. The total leukocyte count also was reduced after each exposure. Mortality in the group exposed at 5000 ppm toluene was 18/25 by the end of the 5-week study. Other tissues, liver, heart, spleen, adrenals and bone marrow, appeared normal in rats treated at both dose levels.

Jenkins et al. (1970) reported that no effects were evident in rats, guinea pigs, dogs or monkeys exposed to toluene vapor. Half of the animals were exposed at 107 ppm (389 mg/m³) toluene continuously for 90 days while the remaining animals were exposed at 1085 ppm (4095 mg/m³), 8 hours/day, 5 days/week for 6 weeks. Parameters used to determine toxicity were leukocyte counts, hemoglobin content, hematocrit and pathology of the liver, kidneys, lungs, spleen, heart, brain and spinal cord. All animals gained weight during the study, except the monkeys (control group for monkeys also did not gain weight). Two of 15 rats exposed continuously at 107 ppm toluene died during the study. These two rats were not examined to determine if toluene exposure was the cause of death. Compared with control groups of rats (n=14), guinea pigs (n=15), dogs (n=10) and monkeys (n=12), histopathological examinations and hematological parameters were "essentially negative" (Jenkins et al., 1970).

Rats exposed to high levels of toluene vapor (12,000 ppm) for seven 10-minute exposures, each followed by a 20-minute nonexposed period, 5 days/week for 8 weeks, had depressed body weight (Bruckner and Peterson, 1981b). Histologically, no effects were evident on liver, lung, kidney, brain or heart, but decreased lung, kidney and brain weights were reported. SGOT and LDH levels were slightly increased in treated rats, while BUN levels were not affected.

Ungvary et al. (1980) reported that SGOT and SGPT levels were not affected in rats treated at 265 ppm (1000 mg/m^3), 6 hours/day, 5 days/week for up to 6 months. The cytochrome P-450 content of the liver was significantly increased ($p<0.01$). In female rats, significantly ($p<0.01$) increased liver-to-body weight ratio was noted, while total body weight was decreased. Although this tendency was evident in male rats exposed to 1000 mg/m^3, the changes in liver weight and body weight were not statistically significant. In male rats treated at 3500 mg/m^3 for 6 months, body weight was depressed and relative liver weight was increased. In rats treated with toluene at 3000 or 6000 mg/m^3 for 8 hours/day on 5 days/week for only 4 weeks, liver-to-body weight ratio was increased significantly ($p<0.01$). Exposure at 1500 mg/m^3 for 4 weeks did not cause an increase in relative liver weight. Hepatic cytochrome P-450 content was increased in rats exposed at all three dose levels but significantly ($p<0.01$) so at the two higher dose levels (Ungvary et al., 1980).

In a 26-week inhalation study performed by Biodynamics for the American Petroleum Institute (1980), groups of Sprague-Dawley rats (15 of each sex) were exposed to toluene at 0, 100 or 1500 ppm (0, 377 or 5650 mg/m^3) for 6 hours/day, 5 days/week. Initially, the high-dose group was exposed to 2000 ppm toluene (for seven exposures) but this level was reduced to alleviate CNS effects. Following analysis of hematologic and clinical chemistry parameters, blood glucose and SGPT levels in female rats were increased in a dose-related fashion, but values were still within normal ranges. No treatment-related histopathological changes or significant changes in blood or urine were reported (American Petroleum Institute, 1980).

Subchronic inhalation exposure to toluene at 900 or 1400 ppm (3390-5280 mg/m^3) for 14 hours/day, 7 days/week for 14 weeks resulted in reduced weight gain and slight behavioral changes in treated male F344 rats (Pryor et al., 1983a). Weanling rats, aged 21 days, were used at the start of the study. Each experimental group, including air-exposed controls, included 11 or 12 rats. Toluene purity was >99% with 0.1% benzene. A battery of behavioral, neuromuscular and neurophysiological tests were performed and three rats of each group were examined histologically for peripheral nerve pathology. Body weight gain was reduced in both toluene-exposed groups after 1 week of exposure. Thereafter, rats exposed to 900 ppm toluene gained weight normally, while those exposed at 1400 ppm continued to show significantly ($p<0.05$) lower body weight than controls. Rats exposed at both levels of toluene showed significant ($p<0.05$) reductions in motor neuron activity (counts/10 minutes) during weeks 2-8 but recovered thereafter. In tone-intensity discrimination tests, where sounds were used to stimulate avoidance behavior, rats treated to 1400 ppm toluene showed a significant ($p<0.05$) reduction in successful avoidances. This factor was due reportedly to hearing loss for lower-frequency sounds. In another study by Pryor et al. (1983b), it was suggested that hearing loss is first evident at high frequencies (12 kHz) and progressively, as dose and exposure are extended, affects mid-range (8 kHz) and low-frequency (4 kHz) hearing.

Pryor et al. (1983a) also noted that learning behavior, determined by a conditioned pole-climb avoidance response, was decreased ($p<0.05$) in rats exposed to 1400 ppm toluene on days 1, 2 and 4 of training and only on day 1 of training in rats treated at 900 ppm. Three stimuli were used to elicit the avoidance behavior, low-level electric shock, 4 kHz sound or a change in chamber light intensity.

In studies with adult mice (6/group) the animals inhaled toluene concentrations of 1, 10, 100 or 1000 ppm (3.77, 37.7, 377 or 3770 mg/m^3) for 6 hours/day on 20 consecutive days (Horiguchi and Inoue, 1977). In mice exposed to 100 or 1000 ppm, cellularity of the bone marrow was decreased and maturation of blood precursors was evident. No histological changes occurred in the liver, kidney, lung, spleen or brain. No definite dose-induced effects were noted in mice treated at 1 or 10 ppm toluene. Groups of 4-6, 5-week-old mice were treated with 12,000 ppm toluene for seven 10-minute exposures/day (10-minute exposures followed by 20 minutes of nonexposure) for 8 weeks (5 days/week). No effects on liver, kidneys, lung, heart or brain were reported (Bruckner and Peterson, 1981b). For mice treated with 4000 ppm (15,080 mg/m^3) toluene (99.9% purity) for 3 hours/day for 8 weeks (5 days/week), body weight was depressed until week 8 and liver weight was increased after week 4 but was not different by week 8 (Bruckner and Peterson, 1981a). No changes in organ histology were evident.

Renal and hepatic degeneration and pulmonary inflammation was evident in guinea pigs treated with toluene at 1250 ppm (4700 mg/m³) by inhalation (4 hours/day, 6 days/week for 3 weeks) (Smyth and Smyth, 1928). Treatment at 1000 ppm (3770 mg/m³) for 6 weeks (4 hours/day, 6 days/week; 35 exposure days) caused slight hepatic and renal degeneration but no pulmonary effects.

Several effects have been reported in dogs treated with toluene vapor (see Table V-2). Fabre et al. (1955) reported that each of two dogs exposed to 7.5 mg/ℓ (7500 mg/m³) for 4 months and then 10.0 mg/ℓ (10,000 mg/m³) for the next 2 months developed paralysis, lung congestion and reduced renal function. The two treated dogs died on days 179 and 180, respectively. Pathologic findings included hemorrhagic liver, hemosiderosis of the spleen, hyperemic glomeruli and other renal lesions.

Based on early reports, Batchelor (1927) and Sessa (1948) demonstrated toxic effects following subchronic s.c. injection of toluene (see Table V-2). Administration of 0.87 g/kg (s.c.) for 21 days to rats (Batchelor, 1927) resulted in a decrease in body weight, hyperplasia of bone marrow and spleen, focal necrosis of the liver, slight swelling of the kidneys, and no effect on the heart, testes or lungs. Sessa (1948) injected guinea pigs with 0.22 g/day (s.c.) for the length of their survival (30-70 days). Degenerative changes in the lungs, kidneys, secondary adrenals, liver and spleen were reported in this abstract.

The only chronic toxicity study on toluene was conducted for 24 months in a total of 960 male and female F344 rats (CIIT, 1980). Toluene of 99.98% purity was administered by inhalation at 30, 100 or 300 ppm (113, 377 or

1130 mg/m³) for 6 hours/day, 5 days/week. Clinical chemistry, hematology and urinalysis testing was conducted after 18 and 24 months of exposure. All parameters measured at the termination of the study (BUN, serum alkaline phosphatase, SGPT, urinary specific gravity, blood, ketones, protein and pH, white and red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) were normal except for a dose-related reduction in hematocrit values in females exposed to 100 and 300 ppm toluene and increased corpuscular hemoglobin concentration in the high-dose females. Increases in body weight evident in treated males and females were not considered treatment-related. Although some inflammatory and degenerative changes were seen in various tissues, control animals also had some pathological changes; therefore, the authors concluded that these changes were not dose-related.

Target Organ Toxicity

Toxic effects of toluene have been reported in the kidney, liver and lungs of exposed animals. Inhalation of toluene vapor causes CNS effects and behavioral toxicity in animals and humans. Mucous membrane irritation is the first effect of high toluene concentrations following inhalation exposure.

Effects on the Kidney. No toxic effects on the kidneys were reported when toluene was administered by oral intubation at 118, 354 or 590 mg/kg/day for 193 days (5 days/week, 138 total doses) (Wolf et al., 1956). Inhalation of toluene at 600-5000 ppm (2260-18,850 mg/m³) for 5 weeks (7 hours/day, 5 days/week) caused the formation of renal casts within the collecting tubules in treated rats. Within 3 weeks of exposure, casts were evident in rats treated at 600 ppm and appeared within 2 weeks at 2500 ppm toluene (von Oettingen et al., 1942a). Batchelor (1927) reported severe clouded swelling of renal tissue upon histological examination of rats treated at 1600 ppm (6030 mg/m^3) toluene for 18-20 hours/day for 3 days. Bruckner and Peterson (1981b) reported a decrease in kidney weight in rats inhaling toluene at 12,000 ppm for seven 10-minute cycles/day (simulating glue sniffing exposure in humans) (see Subchronic and Chronic Toxicity Section). Upon necropsy, however, the kidneys appeared histopathologically normal. In rats exposed to toluene at 30, 100 or 300 ppm (113, 377 or 1130 mg/m^3), chronic progressive nephropathy was reported as a consiste pathology in control and toluene-exposed animals (CIIT, 1980). This and other renal pathologies could not be attributed to toluene exposure.

Renal pathological changes were reported in dogs treated by inhalation to 200-600 ppm (754-2260 mg/m^3) for 1 week (3 days/week, 8 hours/day), then to 400 ppm (1500 mg/m^3) for 1 week (5 days/week, 7 hours/day) and finally to 850 ppm for 1 hour (von Oettingen et al., 1942a). Hyaline casts within the collecting tubules and fat accumulation in the convoluted tubules were observed. Fabre et al. (1955) reported hyperemic renal glomeruli and albumin in the urine of dogs treated with toluene vapor for 6 months (2000 ppm for 4 months, then 2600 ppm for 2 months, 8 hours/day, 6 days/week).

In guinea pigs, slight renal degeneration was evident following 35 exposures (4 hours/day, 6 days/week) at 1000 ppm (3770 mg/m^3) toluene (Smyth and Smyth, 1928). The same researchers noted that 3 weeks of treatment at 1250 ppm (4710 mg/m^3) toluene on the same schedule produced more marked degeneration within the kidney. Sessa (1948) reported in an abstract that s.c. administration of toluene (at 0.22 g/animal/day) for up to 70 days produced degeneration in the convoluted tubule epithelium in guinea pigs.

Effects on the Liver. Toluene administered by oral intubation at 118, 354 or 590 mg/kg/day for 193 days (5 days/week) to rats caused no hepatic pathologic changes (Wolf et al., 1956). Toxic effects of toluene on the liver have been reported in rats treated by inhalation (von Oettingen et al., 1942a). Liver weight and volume were increased in a dose-related manner in rats exposed to 600, 2500 or 5000 ppm (2260, 9430 or 18,850 mg/m³). Histopathologically, hepatocytes stained less intensely (indicative of reduced cytoplasmic density) as the level of toluene exposure increased. Ungvary et al. (1980) reported that hepatocytes of rats treated at 3500 mg toluene/m³ (8 hours/day, 5 days/week) for up to 6 months had enlarged cisternal spaces in the rough endoplasmic reticulum. Activity for a few hepatic enzymes and cytochrome P-450 content was also increased. In rats tested at 3000 and 6000 mg/m³ for 4 weeks (8 hours/day, 5 days/week) relative liver weight and hepatic cytochrome P-450 content were increased (Ungvary et al., 1980). Liver-to-body weight ratio was increased in rats treated to 4000 ppm (15,080 mg/m³) after 4 weeks of exposure but this difference was not evident after 8 weeks (Bruckner and Peterson, 1981b). Smyth and Smyth (1928) reported slight and marked liver degeneration in rats treated at 1000 and 1250 ppm (3770 and 4710 mg/m³) for 35 and 18 exposures, respectively. In the chronic inhalation study on toluene, conducted by CIIT (1980), no toxic effects on the liver were noted in rats exposed to 30, 100 or 300 ppm (113, 377 or 1130 mg/m³). Hemorrhagic liver was reported in two dogs exposed to 2000 ppm (7540 mg/m³) toluene for 4 months followed by 2 months of treatment at 2660 ppm (10,030 mg/m³) (Fabre et al., 1955). Focal necrosis of the liver was reported in rats treated by s.c. injection with toluene (0.87 g/kg) for 21 days (Batchelor, 1927).

Effects on the Lungs. The effect of toluene on the lungs is primarily irritation following inhalation of the vapor. von Oettingen et al. (1942a), however, reported congestion in dogs and pulmonary lesions in rats subchronically exposed by inhalation (see Table V-2). Bruckner and Peterson (1981b) reported decreased lung weights in mice exposed periodically at 12,000 ppm toluene for seven 10-minute exposures/day for 8 weeks (5 days/week). Pulmonary inflammation was reported in guinea pigs exposed to 1250 ppm toluene vapor for 4 hours/day on 6 days/week for 3 weeks (Smyth and Smyth, 1928). In an abstract, Sessa (1948) reported pulmonary hemorrhage, hyperemia and degenerative changes in guinea pigs treated by s.c. injection (0.22 g/animal/day for up to 70 days).

Effects on the CNS. Acute exposure to high levels of toluene induced depression of the CNS. Batchelor (1927) reported that uncoordinated movement and narcosis were produced in rats following inhalation of toluene at 1250 and 1600 ppm (4710 and 6030 mg/m^3) for 18-20 hours. Inhalation of toluene at 1100 ppm (4150 mg/m^3) in rats reportedly did not affect the CNS. Bruckner and Peterson (1981b) noted that the rate and extent of narcosis and CNS depression was dependent on dose in mice exposed to 2600, 5200 or 12,000 ppm (9800, 19,600 or 45,240 mg/m^3). Recovery after cessation of exposure was rapid, occurring within 30 minutes in rats exposed to 12,000 ppm for 20 minutes. Behavioral performance and muscular coordination decreased with increased level and duration of toluene exposure. The decrements in performance were proportional to increasing brain toluene content (Peterson and Bruckner, 1978). The concentration of toluene in the brain increased exponentially with duration and level of exposure and was cleared similarly from the brain after exposure was terminated.

Unconditioned reflexes (corneal and grip) and simple behaviors (righting response, coordination and muscular movement) were adversely affected in male rats exposed to 800 ppm (3020 mg/m^3) toluene for 4 hours (Krivanek and Mullin, 1978). Horiguchi and Inoue (1977) reported that toluene at a level of 1 ppm (3.77 mg/m^3) toluene for 6 hours/day began to decrease the wheel-turning activity in male mice after 10 days of exposure. Faustov (1958) reported that 265 ppm (1000 mg/m^3) toluene was the threshold level of CNS effects in rats and mice treated by inhalation.

Pryor et al. (1983a,b) reported progressive hearing loss, initially high frequency loss, in rats inhaling 900 or 1400 ppm toluene for up to 14 weeks.

Effects on the Hematopoietic System. Before 1940, toluene was believed to have myelotoxic effects similar to that produced by benzene. In fact, in most early studies, the toluene administered to animals was contaminated with benzene, which imparted the myelotoxic effect. Recently, most studies using relatively pure toluene have discounted the effects of toluene on hematopoietic tissues, although a few still indicate a positive effect.

Following oral administration at 118, 354 or 590 mg toluene/kg/day, blood cell and bone marrow counts were normal as were spleen and bone marrow histologies in treated rats (Wolf et al., 1956). von Oettingen et al. (1942a), using toluene relatively free of benzene, demonstrated that blood and blood forming tissues in rats and dogs were not affected by subchronic inhalation of 200-5000 ppm toluene. No effects were reported on spleen or bone marrow and only temporary decreases of blood cell counts occurred. In the chronic 24-month study in which rats inhaled relatively pure toluene at

30, 100 or 300 ppm, no histological changes were seen in the spleen or bone marrow and, except for reduced hematocrit and increased mean corpuscular hemoglobin concentration in females, no effect on several hematological parameters was noted (CIIT, 1980).

In contrast, Horiguchi and Inoue (1977) reported that mice exposed to 1, 10, 100 or 1000 ppm toluene for 20 days showed hematotoxicity. Leukocytosis was evident at all dose levels, while decreased thrombocyte counts were reported in mice treated at 10-1000 ppm. Decreased erythrocyte counts were reported in mice exposed to 100 and 1000 ppm. Based on bone marrow histology, Horiguchi and Inoue (1977) suggested that mice treated at 100 ppm (3770 mg/m^3) toluene showed a trend toward bone marrow hyperplasia.

Other Effects

Carcinogenicity. The only chronic bioassay of toluene, which investigated its tumorigenic and carcinogenic potential in male and female F344 rats, is that conducted by CIIT (1980). Animals (n=960 total) were exposed by inhalation for 6 hours/day, 5 days/week for 24 months to toluene at 0, 30, 100 or 300 ppm. An extensive histopathological examination of tissues and organs was conducted at 24 months on control rats and rats exposed to 300 ppm. Ophthalmologic and clinical changes (hematologic, blood chemistry and urinalysis) also were determined in selected rats of each group. Rats dying during the exposure period were examined for gross pathologies. The results indicate that the incidence of tumors or proliferative lesions was not different between control and toluene-treated rats. A variety of lesions and pathological changes were reported in 300 ppm toluene-treated rats, but the incidences of these lesions or changes were not elevated over control values. Toluene was, therefore, concluded to be noncarcinogenic in F344 rats at the concentrations tested (CIIT, 1980).

Although the study by CIIT (1980) concluded that toluene was a noncarcinogen, Powers (1979) noted that the MTD of a chemical is usually used as a target for the high-dose exposure in carcinogenicity testing. The 300 ppm toluene level used in this 24-month study was not near the MTD. Since there was a high incidence of lesions and pathological chnages in the control F344 rats, additional testing should be conducted with more than one species/strain of test animal. Because of these inadequacies in the data for assessing carcinogenic potential, the available bioassay data is considered inadequate to evaluate the carcinogenic potential.

The National Cancer Institute (NCI, 1983) has initiated a 2-year carcinogenicity bioassay on commercial toluene in which the compound is being administered by inhalation to F344/N rats and B6C3F1 mice. As of January 13, 1987, the quality assessment phase of the 2-year study is in progress. Prechronic testing of toluene, administered by gavage in rats and mice, has also been conducted (NCI, 1983). A technical report of the prechronic testing data is not yet available.

Several studies have examined the carcinogenicity of toluene following repeated dermal applications. Toluene (dose not specified) applied to the shaved interscapular skin of 54 male mice (strains A/He, C3HeB, SWR) throughout their lifetime (3 times weekly) produced no carcinogenic response (Poel, 1963). One drop of toluene (~6 μℓ) applied to the dorsal skin of

20 random-breed albino mice twice weekly for 50 weeks caused no skin papillomas or carcinomas even after a 1-year latency period was allowed (Coombs et al., 1973). No increase in the incidence of skin or systemic tumors was demonstrated in male or female mice of three strains (CF, C_3H or CBaH) when toluene was applied to the back of ~25 mice of each sex of each strain at 0.05-0.1 mℓ/mouse, twice weekly for 56 weeks (Doak et al., 1976). One skin papilloma and a single skin carcinoma were reported among a group of 30 mice treated dermally with one drop of 0.2% (w/v) solution toluene, twice weekly administered from droppers delivering 16-20 μℓ per drop for 72 weeks (Lijinsky and Garcia, 1972). It is not definite whether evaporation of toluene from the skin was prevented during these studies.

In two studies, conducted by Frei and Stephens (1968) and Frei and Kingsley (1968), toluene was reported as having little to no promoting activity after initiation with 7,12-dimethylbenz(alpha)anthracene (DMBA). Swiss mice were treated on the ears or the shaved back with a single 0.1 mℓ treatment of 1.5% DMBA in mineral oil followed 1 week later by twice weekly applications of 0.1 mℓ toluene for 20 weeks. Experimental groups in the two studies had 11/35 and 7/35 skin tumors, while DMBA-only treated mice developed 8 among 53 animals surviving 20 weeks. Of the 14 mice treated with toluene only, 2 tumors (1 permanent and 1 regressive) developed.

Mutagenicity.

Assays Using Microbial Species -- Toluene has been tested for mutagenic activity by several investigators (Mortelmans and Riccio, 1980; Nestmann et al., 1980; Bos et al., 1981; Litton Bionetics, Inc., 1981; Snow et al., 1981) in the Ames *Salmonella typhimurium* assay in tester strains

TA98, TA100, TA1535, TA1537 and TA1538. In all cases, toluene was found not to be mutagenic (Table V-3). All assays were conducted in the presence and absence of a rat liver S-9 homogenate (Aroclor 1254-induced) with appropriate positive and negative controls. Snow et al. (1981) conducted tests using a toluene-induced rat liver homogenate as well. Evaporation of toluene may have occurred in all studies except that of Snow et al. (1981), which stated that assays were conducted in sealed chambers. Toluene was also not effective in increasing the reversion of the Escherichia coli strain WP2 to tryptophan-independence (Mortelmans and Riccio, 1980).

The reversion of the eukaryotic yeast, Saccharomyces cerevisiae strain D7, to isoleucine independence was not increased by toluene, with or without an activation system (Mortelmans and Riccio, 1980). Toluene did not induce gene conversions to tryptophan independence in S. cerevisiae strains D4 (Litton Bionetics, Inc., 1981) and D7 (Mortelmans and Riccio, 1980). Mitotic crossing-over at the ade-2 locus was not induced by toluene in S. cerevisiae strain D7 (see Table V-3) (Mortelmans and Riccio, 1980).

Damage to DNA was not evident using wild type and DNA repair-deficient strains of E. coli (W3110, pol A^+; p3478, pol A^-) (Fluck et al., 1976) and S. typhimurium (SL4525, rfa rec^+; SL4700, rfa rec^-) (Mortelmans and Riccio, 1980). Fluck et al. (1976) applied toluene at 25 µℓ/plate into wells in the culture plates, while Mortelmans and Riccio (1980) used filter discs inoculated with 0.001-0.01 µℓ toluene. Growth inhibition was not shown in either study, in the presence or absence of an activation system. No difference was noted between wild type and DNA repair-deficient strains, indicating a lack of DNA damage.

TABLE V-3

Microbial Mutagenicity Assays

Type of Assay	Strain	Metabolic Activation[a]	Dose	Application	Response	Reference
Reverse Mutation						
S. typhimurium	TA98, 100, 1535, 1537, 1538	yes and no	0.001-5.0 µl/plate	plate incorporation	-	Litton Bionetics, Inc., 1981
S. typhimurium	TA98, 100, 1535, 1537, 1538	yes and no	0.004-0.031%[b]	liquid suspension	-	Litten Bionetics, Inc., 1981
S. typhimurium	TA98, 100, 1535, 1537, 1538	yes and no	0.01-10 µl/plate	plate incorporation	-	Mortelmans and Riccio, 1980
S. typhimurium	TA98, 100, 1535, 1537, 1538	yes and no	5 µl/plate	plate incorporation	-	Westmann et al., 1980
S. typhimurium	TA98, 100, 1535, 1537, 1538	yes and no	0.115-2.3 µl/plate	plate incorporation	-	Bos et al., 1981
S. typhimurium	TA98, TA100	yes and no[c] yes and no[c]	0.3-100 µl/plate 11-3764 ppm	plate incorporation[d] vapor exposure[e]	- -	Snow et al., 1981
E. coli	WP2	yes and no	0.01-10 µl/plate	plate incorporation	-	Mortelmans and Riccio, 1980
S. cerevisiae	D7	yes and no	0.001-0.5%[f]	liquid suspension	-	Mortelmans and Riccio, 1980

TABLE V-3 (cont.)

Type of Assay	Strain	Metabolic Activation[a]	Dose	Application	Response	Reference
Mitotic Gene Conversion						
S. cerevisiae	D4	yes and no yes and no	0.001-5.0 µl/plate 0.138-1.1%[b]	plate incorporation liquid suspension	- -	Litton Bionetics, Inc., 1981
S. cerevisiae	D7	yes and no	0.001-0.5%[f]	liquid suspension	-	Mortelmans and Riccio, 1980
Mitotic Crossing-Over						
S. cerevisiae	D7	yes and no	0.001-0.5[f]	liquid suspension	-	Mortelmans and Riccio, 1980

[a] Aroclor 1254-induced rat liver homogenate S-9 fraction

[b] 50% mortality at the highest dose

[c] The toluene was tested with toluene-induced S-9 as well as with Aroclor induced S-9.

[d] The plates were incubated in sealed plastic bags or chambers for part of a 72-hour incubation period; in the Aroclor-induced S-9 tests, the plates were removed from the bags after 48 hours, counted, incubated an additional 24 hours, and recounted; in the experiments with toluene-induced S-9, the plates were removed after 24 hours to prevent moisture and spreading problems, and then incubated an additional 48 hours before counting.

[e] The assays were run in a sealed incubation chamber with a second glass plate (open) that contained the toluene; after 24 hours the chambers were opened and the plates incubated for an additional 48 hours.

[f] 100% mortality at 0.1% and 0.5%

Assays Using Nonhuman Mammalian Species -- The effect of toluene on the incidence of chromosomal aberrations in bone marrow cells was determined in Charles River rats injected i.p. with the pure compound (Litton Bionetics, Inc., 1981). Toluene was injected at 22, 71 or 214 mg/kg for one dose or on 5 consecutive days. In the 1-day study, rats were sacrificed at 6, 24 and 48 hours postexposure, while all five rats treated for 5 days were sacrificed 6 hours after the last treatment. Solvent (DMSO) controls and positive controls (triethylenemelamine) were used for comparison. The results of the bone marrow cell analysis (Table V-4) indicate that toluene treatment, at any level for 1 or 5 days of exposure, did not cause an increase in the frequency or type of chromosomal aberration.

In contrast to the Litton Bionetics, Inc. (1981) study, which reported that toluene did not cause increased chromosomal aberrations in bone marrow cells, several Russian studies (evaluation based on English translation) report toluene as being effective in causing chromosomal damage in bone marrow cells. After analyzing 720 bone marrow cells in metaphase (derived from five rats injected s.c. with 0.8 g toluene/kg/day for 12 days), Dobrokhotov (1972) found that 13% (78/720) showed chromatid aberrations. The frequency of aberrations among 600 marrow cells derived from vehicle-injected rats was ~4%. Aberrations in toluene-exposed cells included chromatid breaks, chromatid fractures, chromosome fractures and, occasionally, multiple aberrations, while control-derived cells had no fractures or multiple aberrations. Similarly, Lyapkalo (1973) reported 84 of 724 (11.6%) bone marrow cells derived from six rats treated with 1 g toluene/kg/day (s.c. injection) for 12 days had chromatid and chromosome aberrations. Comparatively, cells derived from vehicle (olive oil) control rats had 3.87%

TABLE V-4

Rat Bone Marrow Cell Aberrations Following Intraperitoneal Injection of Toluene[a]

Treatment[b]	Dose	Time of Sacrifice	No. of Animals	Total No. of Cells	Type and Frequency of Aberrations		No. of Cells With One or More Aberrations	No. of Animals Without Aberrations	Mitotic Index[d]
					Structural[c]	Numerical			
DMSO (solvent)	0.65 mL/rat	6 hours	5	225	2f,1td	ND	3 (1.3%)	3	3.8
		24 hours	5	250	ND	ND	0 (0.0%)	5	6.0
		48 hours	5	250	1tb,1f	ND	2 (0.8%)	4	6.1
		6 hours (SA)[e]	5	227	1td	ND	1 (0.4%)	4	5.0
Triethylene-melamine	0.3 mg/kg	24 hours	5	250	11tb,2sb,5af,45f, 26t,1r,10td,12>, 1pu,1qr,2ac,3tr	2pp	72 (28.8%)	0	1.4
Toluene	2 mg/kg	6 hours	5	250	ND	ND	0 (0.0%)	5	3.4
		24 hours	5	242	ND	ND	0 (0.0%)	5	5.9
		48 hours	5	250	ND	ND	0 (0.0%)	5	7.0
		6 hours (SA)	5	238	3f	ND	2 (0.8%)	3	6.3
Toluene	1 mg/kg	6 hours	5	239	1td	1pp	2 (0.8%)	4	2.5
		24 hours	5	227	2td,1af,1f	ND	4 (1.8%)	3	4.3
		48 hours	3	150	ND	ND	0 (0.0%)	3	5.7
		6 hours (SA)	5	212	ND	ND	0 (0.0%)	5	3.3
Toluene	4 mg/kg	6 hours	5	250	1f	2pp	3 (1.2%)	3	4.5
		24 hours	5	250	ND	1pp	1 (0.4%)	4	3.6
		48 hours	5	250	1tb,1td	ND	2 (0.8%)	3	5.4
		6 hours (SA)	5	250	1td,3af	ND	2 (0.8%)	3	5.4

[a] Source: Adapted from Litton Bionetics, Inc., 1981

[b] The toluene used was 99.96 wt. % pure (ethylbenzene, 0.03%; p-xylene, <0.01%; m-xylene, <0.01%; sulfur, 0.4 ppm) (Fowle, 1981)

[c] af - acentric fragment (2 ltd); f - fragments; pp - polyploid; pu - pulverized chromosome; qr - quadriradial; r - ring; sb - chromosome break; t - translocation; tb - chromatid break; td - chromatid deletion; tr - triradial; > - greater than 10 aberrations

[d] Based on a count of at least 500 cells per animal

[e] SA - subacute study; rats were dosed daily for 5 days, with sacrifice 6 hours after the last dose

ND - Not detected

(40/1033) aberrations. Bone marrow cells derived from rats treated with 80 ppm (300 mg/m³) toluene by inhalation for 4 months (4 hours/day) had an aberration frequency of 21.6% when examined in metaphase (Dobrokhotov and Yenikeyeu, 1975). Chromosomal aberrations in bone marrow cells derived from air-exposed rats occurred in 4.0% of those examined (the number of cells examined in controls or toluene-exposed groups was not reported). In all three of these Russian studies (Dobrokhotov, 1972; Lyapkalo, 1973; Dobrokhotov and Yenikeyeu, 1975), the purity of the toluene was not reported and may have been contaminated with benzene. All three studies showed that benzene was effective in causing chromatid aberrations in bone marrow cells.

Assays in Humans -- Peripheral blood lymphocytes have been used almost exclusively to study chromosomal aberrations and sister-chromatid breaks in exposed humans. Forni et al. (1971) analyzed lymphocyte chromosomes for stable (deletions, translocations, trisomies) and unstable (fragments, dicentric or ring chromosomes) chromosomal changes among 10 workers exposed to benzene and toluene and 24 toluene-only exposed workers. A group of 34 matched controls not exposed to either solvent was used for comparison. Ten workers were exposed to benzene at 131-532 ppm for 2-7 years and subsequently, 200-400 ppm toluene for 14 years. The remaining 24 workers were exposed to toluene (200-400 ppm) for 7-15 years. The frequency of stable or unstable chromosomal abnormalities was not increased in workers exposed only to toluene, compared with matched controls. Workers exposed to benzene and toluene followed by toluene only had increased numbers of stable and unstable aberrations compared with the matched controls and the toluene-only exposed workers (Table V-5) (Forni et al., 1971).

TABLE V-5

Frequency of Unstable and Stable Chromosome Changes and Chromosome Counts in Male Subjects Exposed to Benzene or Toluene or Both[a]

Exposure Subjects	No. of Cases	Age Range	Total Cells Counted	Percent Cells		Percent Cells with Chromosome Number		
				C_u[b]	C_s[c]	<46	46	>46 (polyploid)
Benzene (+ toluene)	10	36-54	964	1.66 (1.87)[d,e,f]	0.62[e,f]	13.1	86.0	0.9 (0.52)
Toluene	24	29-60	2400	0.80 (0.83)[d]	0.08	14.3	85.4	0.3 (0.29)
Control subjects	34	25-60	3262	0.61 (0.67)	0.09	10.2	89.5	0.3 (0.3)

[a] Source: Adapted from Forni et al., 1971

[b] Cells with "unstable" chromosome aberrations (fragments, dicentrics, ring chromosomes). The presence of each fragment was considered as one break, the presence of a dicentric or ring chromosome as two breaks.

[c] Cells with "stable" chromosome changes (abnormal monocentric chromosomes due to deletions, translocations, etc., trisomies)

[d] Numbers in parentheses show percentage of calculated breaks.

[e] Difference from toluene group was significant ($p<0.05$)

[f] Difference from control was significant ($p<0.01$)

In a study of lymphocyte chromosomes from 22 female laboratory workers in a rotogravure plant, Funes-Craviota et al. (1977) noted a slight increase in aberrations in workers exposed to toluene and benzene. Fourteen workers were thought to be exposed to toluene only (100-200 ppm with occasional 500-700 ppm levels) for 1.5-26 years, but may have been exposed to benzene as a contaminant. These workers showed a slight increase in chromosomal aberrations compared with a group of 49 controls (Table V-6). Eight workers exposed to benzene (for 2-10 years) before exposure to toluene (up to 26 years) had a higher frequency of chromosomal abnormalities than the toluene-only group or the controls.

Recently, Maki-Paakkanen et al. (1980) studied a group of 32 printers and assistants who were exposed to pure toluene (average 0.006% benzene concentration) at 7-112 ppm (TWA concentrations) for 8 hours daily. A comparison of the incidence of chromosomal aberrations and sister chromatid exchanges was made with a group of 15 unexposed research institute workers (Table V-7). No significant differences in the incidence of chromatid gaps, chromosome gaps or sister chromatid exchanges were evident between toluene-exposed and control derived lymphocytes. A breakdown based on duration of toluene exposure did not show a relation between chromosome abnormalities and exposure duration. A significant increase in the incidence of sister chromatid exchanges was evident among smokers versus nonsmokers in exposed and control ($p<0.01$) groups (Maki-Paakkanen et al., 1980).

Cultured human lymphocytes exposed to toluene at 1.52, 152 or 1520 µg/ml *in vitro* did not have an elevated number of chromosomal aberrations or sister chromatid exchanges (Gerner-Smidt and Friedrich, 1978).

TABLE V-6

Chromosome Aberrations in Rotoprinting Factory Workers[a]

	Group[b]		
	Control	Toluene	Benzene/Toluene
No. of Subjects (sex not reported)	49	14	8
Age (year)			
Range	0.16-63	23-54	54-65
Mean	24.4	37.2	61.3
No. of Cells Analyzed			
Total	5000	1400	800
Abnormal			
Total	217	108	76
Frequency range (%)	0-20	2-15	4-17
Mean frequency (%)	4.3	7.7	9.5
No. of Chromosomes Analyzed			
Total	230,000	64,400	36,800
Breaks			
Total	233	124	95
Range (per 100 cells)	0-22	2-17	6-17
Mean (per 100 cells)	5.1	8.9	11.9

[a] Source: Adapted from Funes-Craviota et al., 1977

[b] Exposure details provided in accompanying text

TABLE V-7

Effect of Occupational Toluene Exposure and Smoking on Chromosomal Aberrations and Sister Chromatid Exchanges[a]

Occupational Toluene Exposure (years)	No. of Subjects[b]	Mean Age (years)	Cells Analyzed[c]	Cells with Chromosomal Aberrations (%)				Sister Chromatid Exchanges	
				Gaps Excluded			Gaps Included Total		
				Chromatid Type	Chromosome Type	Total		Cells Analyzed[d]	Mean per Subject per Cell[e]
Total Worker (14.6 years average exposure)	32	34.2[f]	3200	1.0	0.5	1.5	2.5	909	8.5
Total Control	15	34.2[f]	1900	0.7	0.9	1.6	2.7	552	8.9
0 (controls)									
Nonsmokers	4	31.0	800	0.5	0.8	1.3	2.3	234	8.0
Smokers	11	35.5	1100	0.9	1.0	1.8	3.1	318	9.7 (p<0.01)
Total	15	34.3	1900	0.7	0.9	1.6	2.7	552	9.2
1-10 (mean, 8.0)									
Nonsmokers	3	27.7	300	0.7	0.3	1.0	2.3	79	7.9
Smokers	10	26.2	1000	0.7	0.3	1.0	1.9	295	9.1 (p<0.001)
Total	13	28.1	1300	0.7	0.3	1.0	2.0	374	8.8
>10 (mean, 19.3)									
Nonsmokers	11	38.5	1100	0.8	0.5	1.4	2.5	330	7.5
Smokers[g]	8	35.9	800	1.8	0.8	2.5	3.1	205	9.6 (p<0.001)
Total	19	37.5	1900	1.2	0.6	1.8	2.8	535	8.3

[a] Source: Adapted from Maki-Paakkanen et al., 1980

[b] all subjects were males

[c] ~100 cells analyzed per individual

[d] ~30 cells analyzed per individual

[e] Calculated from individual means

[f] Mean value

[g] Sister chromatid exchanges were analyzed from seven subjects: p<0.01 and p<0.001 compared with nonsmokers in the group, one-tailed student's t-test

In these studies, only 26 cells/dose were analyzed for aberrations, and tests using a mammalian activation system were not performed. A significant ($p<0.05$) inhibition of growth was reported in cells treated at the two higher levels of toluene.

Teratogenicity. The only available data on the teratogenic potential of toluene following oral administration is that reported in an abstract by Nawrot and Staples (1979). Toluene (purity not specified) was administered to pregnant CD-1 mice at 0.3, 0.5 and 1.0 mℓ/kg/day (0.26, 0.43 and 0.87 g/kg/day) in cottonseed oil by gavage on days 6-15 of gestation or at 1.0 mℓ/kg/day (0.87 g/kg/day) on days 12-15 of gestation. A significant increase in embryo death was reported in all groups treated with toluene on days 6-15. Time of fetal examination was not reported. Fetal weight was reduced in groups treated at 0.5 and 1.0 mℓ/kg/day on days 6-15 of gestation. The incidence of cleft palate was increased in fetuses exposed to 1.0 mℓ toluene/kg/day on days 6-15 of development and these malformations did not appear to be due solely to slowed growth rate. Maternal toxicity (decreased weight gain) was the only effect noted in mice treated on days 12-15 of gestation. Maternal toxicity was not seen at any dose when exposure was on days 6-15. These results are only preliminary, since they appear only in an abstract and quantitative data with statistical analyses of the "teratogenic effect" are not available. To date, a complete report of this study (Nawrot and Staples, 1979) has not been published in the open literature for critical review.

Toluene was tested for teratogenic potential following inhalation exposure of CFLP mice and CFY rats (Hudak and Ungvary, 1978). Mice were exposed at 500 or 1500 mg/m^3 for 24 hours/day on days 6-13 of gestation. Rats were exposed at 1000 mg/m^3 for 8 hours/day on days 1-21 of gestation, at 1500 mg/m^3 for 24 hours/day on days 1-8, or at 1500 mg/m^3, 24 hours/day on days 9-14 of gestation. All mice exposed at 1500 mg/m^3 died within 24 hours. Low fetal weights, indicating embryotoxicity, were noted in mice treated at 500 mg/m^3 (Table V-8). In rats treated at 1500 mg/m^3, maternal survival was decreased (7/35 mortality ratio). Retarded skeletal development, which resulted in poorly ossified sternebrae, split vertebral centra and shortened free-ribs, was evident in rats treated at 1000 mg/m^3 and those treated at 1500 mg/m^3 on days 1-8 of development. The skeletal anomalies, extra ribs and fused sternebrae were reported in fetuses of rats exposed to 1500 mg/m^3 on days 9-14 of gestation. No increases in visceral or external malformations in mice or rats were identified (see Table V-8) (Hudak and Ungvary, 1978).

In a study comparing the teratogenicity of toluene, benzene or a combination of the two solvents, Tatrai et al. (1980) exposed groups of 20 CFY rats for 24 hours/day on days 7-14 of gestation. Toluene was administered at 1000 mg/m^3 and a group of 22 rats inhaling air served as controls. Similar to data reported by Hudak and Ungvary (1978), Tatrai et al. (1980) reported that skeletal growth retardation and extra ribs were the prominent effects of toluene exposure during fetal development (Table V-9). No internal or external malformations were reported. Maternal relative liver weight was increased slightly ($p<0.05$) above controls. Exposure to toluene did not significantly affect maternal or fetal survival or body weight.

TABLE V-8

Teratogenicity Evaluation of Toluene in CFY Rats and CFLP Mice[a]

	Rats					Mice		
	Air Inhalation	Toluene		Air Inhalation	Toluene	Air Inhalation	Toluene	
	Days 1-21 8 hours/day	1000 mg/m³ Days 1-21 8 hours/day	1500 mg/m³ Days 1-8 24 hours/day	Days 9-14 24 hours/day	1500 mg/m³ Days 9-14 24 hours/day	Days 6-13 24 hours/day	500 mg/m³ Days 6-13 24 hours/day	1500 mg/m³ Days 6-13 24 hours/day
No. pregnant animals examined	10	10	9	26	19	14	11	NA
No. pregnant animals died	0	0	5	0	2	0	0	15
Maternal weight gain[b] (%)	46.6	44.1	44.0	46.9	41.8	NR	NR	NA
No. live fetuses	111	133	95	348	213	124	112	NA
No. resorbed fetuses	8	3	6	15	18	6	10	NA
No. dead fetuses	0	0	0	0	0	1	0	NA
Fetal loss (%)	6.7	2.2	5.9	4.1	7.8	6.1	8.2	NA
Mean litter size	11.1	13.3	10.6	13.4	11.2	9.0	10.2	NA
Mean fetal weight (g)	3.8	3.6	3.3[c]	3.8	3.8	1.1	1.0[c]	NA
Mean placental weight (g)	0.5	0.5	0.5	0.5	0.5	NR	NR	NA
Weight retarded fetuses[d] (%)	7.2	16	46[e]	6.9	17.3	6.5	27.6[e]	NA
External malformations[f]	0	0	0	0	0	0	0	NA
No. fetuses dissected[g]	54	64	49	179	110	64	58	NA
Internal malformations[h]								
Anophthalmia	0	0	0	1	0	0	0	NA
Hydrocephalus	0	0	4	0	0	0	0	NA
Hydronephrosis	1	6	4	16	4	1	3	NA
No. of Alizarin-stained fetuses	57	69	42	169	102	60	54	NA

TABLE V-8 (cont.)

	Rats					Mice		
	Air Inhalation	Toluene		Air Inhalation	Toluene	Air Inhalation	Toluene	
	Days 1-21 8 hours/day	1000 mg/m^3 Days 1-21 8 hours/day	1500 mg/m^3 Days 1-8 24 hours/day	Days 9-14 24 hours/day	1500 mg/m^3 Days 9-14 24 hours/day	Days 6-13 24 hours/day	500 mg/m^3 Days 6-13 24 hours/day	1500 mg/m^3 Days 6-13 24 hours/day
Skeletal retardation signs[i]	0	17[e]	7[e]	11	24[e]	3	1	NA
Skeletal anomalies								
Fused sternebrae	0	0	0	2	7[e]	0	0	NA
Extra ribs	0	0	0	0	22[j]	0	0	NA
Skeletal malformations[k]								
Missing vertebrae	0	0	0	0	2	0	0	NA
Brachimelia	0	0	0	0	0	1	0	NA

[a] Source: Adapted from Hudak and Ungvary, 1978

[b] Percent of starting body weight

[c] $p<0.01$ (t-test)

[d] Percent of living fetuses weighing <3.3 g (rats) or 0.9 g (mice)

[e] $p<0.05$ (Mann Whitney U Test)

[f] Agnathia, brachimelia, missing tail

[g] The rats were sacrificed on day 21 of pregnancy, the mice on day 18

[h] Thymus hypoplasia also looked for

[i] Including poorly ossified sternebrae, bipartite vertebra centra, and shortened 13th ribs

[j] $p<0.01$ (Mann Whitney U Test)

[k] Fissura sterni and agnathia also looked for

NR - Not reported; NA - Not applicable; all dams treated at this level died before examination

TABLE V-9

Teratogenic Effects of Exposure to Toluene, Benzene and a Combination of Toluene and Benzene in CFY Rats[a]

Inhalation on Days 7-14 of Pregnancy 24 hours/day	Air	Toluene 1000 mg/m³	Benzene 400 mg/m³	Toluene/Benzene 1000 mg/m³ 400 mg/m³
Number of females				
treated	21	20	20	20
nonpregnant	1	2	3	1
Number of litters	21	18	17	19
Mean implantation/dam	14.0	14.4	14.6	13.8
Maternal weight gain in percent of starting body weight	68.82 ±2.40[c]	65.82 ±2.13[c]	46.74[b] ±2.69[c]	53.94[b] ±1.84[c]
Relative liver weight (%)	4.25 ±0.08[c]	4.37[d] ±0.07[c]	4.67[d] ±0.12[c]	4.10 ±0.09[c]
Mean placental weight (g)	0.58 ±0.006[c]	0.60 ±0.006[c]	0.48[b] ±0.006[c]	0.54[b] ±0.004[c]
Number of fetuses	94	59	48	62
live	80	39	36	34
dead	0	0	2	0
resorbed	14	20	10	28
Mean fetal weight (g)	3.94 ±0.02[c]	3.91 ±0.02[c]	3.16[b] ±0.03[c]	3.79[e] ±0.02[c]
Weight retarded fetuses in percent of living fetuses	2.8	3.3	57.6[e]	9.8[d]
External malformations	NR	NR	NR	NR
Fetal loss/total implantation sites (%)	4.7	7.7	4.8	10.7[d]
No. Alizarin-stained fetuses	42	21	22	18

TABLE V-9 (cont.)

Inhalation on Days 7-14 of Pregnancy 24 hours/day	Air	Toluene 1000 mg/m³	Benzene 400 mg/m³	Toluene/Benzene 1000 mg/m³ 400 mg/m³
Skeletal retarded fetuses in percent of Alizarin-stained fetuses	13	31[d]	77[b]	39[d]
Skeletal anomalies				
sternum misaligned	4	4	5	1
asymmetric vertebra	1	NR	3	1
extra ribs	1	7[f]	1	19[e]
No. fetuses dissected	38	18	14	16
Internal malformations				
polycystic lungs	1	NR	NR	NR
pyelectasia	2	5	NR	1
dystopia renis	NR	1	NR	NR
vesica giganta	NR	3	1	1
microphthalmia	NR	NR	NR	1
anophthalmia	NR	NR	2	NR
hydrocephalus internus	NR	NR	3	NR

[a] Source: Adapted from Tatrai et al., 1980

[b] $p<0.001$

[c] SEM

[d] $p<0.05$

[e] $p<0.01$

[f] $p<0.1$

NR = None reported

In comparison, exposure to benzene (alone or with toluene) caused decreased body weight in maternal and fetal rats (Tatrai et al., 1980).

No teratogenic effects were reported in fetuses of Charles River rats exposed to toluene by inhalation at 100 or 400 ppm (377 or 1500 mg/m^3) for 6 hours/day on days 6-15 of gestation (Litton Bionetics, Inc., 1978). The incidence of visceral or skeletal anomalies was not different between treated and control groups (Table V-10). Skeletal abnormalities occurred in comparable numbers in both exposed and control rats. The only notable feature was that the entire litter of one female exposed to 400 ppm toluene was resorbed (see Table V-10). Maternal and fetal toxicity was not apparent (Litton Bionetics, Inc., 1978).

The effects of toluene (no impurities) exposure by inhalation at 100 or 1000 ppm were determined pre- and postnatally in mice (Shigeta et al., 1982). Female ICR strain mice, having vaginal plugs, were treated at the two dose levels 6 hours/day on days 1-17 of gestation. Eighteen mice were treated at 100 ppm toluene, 14 at 1000 ppm toluene, and 15 air-exposed mice served as controls. On day 18 of gestation, ~67% of each experimental group was sacrificed with fetuses and uteri examined for abnormalities. The remaining mice delivered and postnatal growth was assessed. No gross embryotoxic or teratogenic effects were evident. The number of resorbed embryos was increased in the treated mice, but not statistically so and not in a dose-related manner. Skeletal abnormalities including extra or rudimentary 14th ribs, fused ribs, cleft vertebrae, cleft sternum, cranioschisis and polydactyly were not increased significantly in any exposure group compared with controls. Parameters used to determine development of the

TABLE V-10

Teratogenicity and Reproductive Performance Evaluation in Rats Exposed to Toluene[a]

	Dose (ppm)		
	0	100	400
Pregnancy ratio (pregnant/bred)	26/27	27/27	27/27
No. pregnant rats that died	0	0	0
Live litters	26	27	26
Implantation sites (left horn/ right horn)	152/194	181/177	179/190
Resorptions	26	28	41[b]
Litters with resorptions	13	20	17
Dead fetuses	0	1	0
Litters with dead fetuses	0	1	0
Live fetuses/implantation site	320/346	329/358	328/369
Mean live litter size (fetuses)	12	12	12
Average fetal weight (g)	3.6	3.5	3.8
Number of fetuses examined for soft tissue (visceral) changes[c]	108(51/57)	105(47/58)	104(51/53)
Number of fetuses examined for skeletal changes[d]	212	221	224
Number of fetuses with normal skeletal examinations	139	150	158
Fetuses with commonly encountered skeletal changes[e,f]	67(20)	62(20)	58(20)
Fetuses with unusual skeletal variations[f,g]	6(4)	9(4)	8(6)

---

[a] Source: Adapted from Litton Bionetics, Inc., 1978

[b] The increase in total resorptions at this dose was attributed to the total resorption of the litter of one particular female.

[c] Numbers of male/females examined in parentheses.

[d] Four specimens from one litter were not examined (missing).

[e] A qualitative examination of the observations recorded for the fetuses indicates that bilateral ribs, unilateral ribs and reduced ossification of various bones were the most frequently encountered changes.

[f] Number of litters in parentheses.

[g] These were generally cases of more severe and extensive retarded ossification.

neonates were time of eye-opening, ear-opening, weaning, body hair growth and coordination of walking. No differences between neonates of the three groups were evident. No difference was evident in body weight of the neonates at weeks 1, 2, 3, 4 or 14 weeks of age (Shigeta et al., 1982).

Summary

The oral toxicity of toluene is relatively low, with an LD_{50} near 7.0 g/kg in adult rats. The earliest observable sign of acute toluene toxicity is inhibition of the functions of the CNS, which became evident at ~2.0 g toluene/kg following oral administration (Kimura et al., 1971). The LC_{50} for inhaled toluene, the predominant route of exposure, was 4618 ppm (17,400 mg/m³) after a 6-hour exposure in rats (Bonnet et al., 1982). No effects were reported after acute exposures at 620 or 1100 ppm (2340 or 4150 mg/m³) toluene, but 1250 ppm (4710 mg/m³) affected coordination and irritated mucous membranes in rats. The dermal LD_{50} in rabbits was 12.2 g/kg (Smyth et al., 1969a).

Following subchronic oral administration, Wolf et al. (1956) reported a NOEL for toluene in female rats to be $\geq$590 mg/kg/day. Following inhalation of toluene for 6 weeks, slight pulmonary irritation was evident in rats exposed at 200 ppm (754 mg/m³) for 7 hours/day, 5 days/week. Renal effects were evident in rats treated at 600 ppm (2260 mg/m³) for 7 hours/day, 5 days/week for 6 weeks (von Oettingen et al., 1942a). Based on the one chronic (24-month) study in F344 rats exposed to 30, 100 or 300 ppm (113, 377 or 1130 mg/m³) toluene, CIIT (1980) concluded that no pathologic changes were dose-related but reported reduced hematocrit values in females exposed at 100 and 300 ppm and increased corpuscular hemoglobin concentration in females exposed at 300 ppm.

The first effect of toluene following inhalation exposure is mucous membrane irritation. Renal effects and effects on the CNS appear to be the major signs of toluene toxicity. Effects also have occasionally been reported in the liver, lungs and hematopoietic tissue.

The only long-term bioassay of toluene concluded that it is not carcinogenic following inhalation in F344 rats (CIIT, 1980). Even though extensive histopathological examinations identified no increase in tumors among treated animals, this study is not adequate for fully evaluating carcinogenicity. The highest dose used in this study (300 ppm) did not approach the MTD and, therefore, it has been suggested that toluene may not have been appropriately tested for carcinogenicity (Powers, 1979). The National Cancer Institute (NCI, 1983) is currently conducting carcinogenicity bioassays in rats and mice exposed to toluene by inhalation. Toluene has been tested for genotoxicity by many investigators using various assay methods and has been, fairly universally, considered nongenotoxic. Based on data reported in an abstract on the oral administration of toluene to mice (Nawrot and Staples, 1979), toluene may induce cleft palates. This was a preliminary report, however, and sufficient data were not available for adequate assessment. Teratogenicity studies, in which toluene was administered by inhalation, indicate that toluene exposure (at 1000 mg/m^3 in rats) results in fetotoxicity and delayed skeletal development but does not cause internal or external malformations.

VI. HEALTH EFFECTS IN HUMANS

Toluene exposure in humans occurs primarily by inhalation, through either deliberate glue sniffing or by occupational exposure. Studies on the toxicity of toluene to humans have been predominantly acute experimental studies or studies of chronically exposed workers.

Glue sniffing usually entails the inhalation of a mixture of volatile organic solvents in glues, thinners or cleaning solvents. Toluene has often been implicated as the effective component causing the intoxicating effects. Toluene is inhaled generally at very high levels for short intervals, with repeated brief exposures. Subchronic or chronic inhalation of toluene vapor may cause psychological dependence and physiological tolerance.

It is important to note that glue sniffing and occupational exposures to toluene usually involve exposure to a mixture of volatile hydrocarbons. Before the mid-1950s, even experimental studies, unless specifically noted, may have used toluene containing benzene (as much as 20%) as a contaminant (Banfer, 1961). In evaluating the effects of toluene, its purity and the possible effects of benzene must also be considered.

Effects of Acute Exposure

The effect of greatest concern following acute exposure to toluene vapor is alteration of CNS function. von Oettingen et al. (1942a,b) reported the effects of toluene vapor (<0.01% benzene) following its inhalation for 8 hours (Table VI-1). Three subjects were exposed twice weekly to toluene vapor at 0, 50, 100, 200, 300, 400, 600 or 800 ppm over an 8-week period. Two exposures/week were used to allow sufficient recovery between exposure

TABLE VI-1

Effects of Controlled 8-Hour Exposures to Pure Toluene on Three Human Subjects[a,b]

Concentration	No. of Exposures	Effects
0 ppm (control)	7	No complaints or objective symptoms, except occasional moderate tiredness toward the end of each exposure, which was attributed to lack of physical exercise, unfavorable illumination, and monotonous noise from fans.
50 ppm (189 mg/m³)	2	Drowsiness with a very mild headache in 1 subject. No after-effects.
100 ppm (377 mg/m³)	4	Moderate fatigue and sleepiness (3), and a slight headache on one occasion (1).
200 ppm (754 mg/m³)	3	Fatigue (3), muscular weakness (2), confusion (2), impaired coordination (2), paresthesia of the skin (2), repeated headache (1), and nausea (1) at the end of the exposure. In several instances, the pupils were dilated, pupillary light reflex was impaired, and the fundus of the eye was engorged. After-effects included fatigue, general confusion, moderate insomnia, and restless sleep in all 3 subjects.
300 ppm (1130 mg/m³)	2	Severe fatigue (3), headache (2), muscular weakness and incoordination (1), and slight pallor of the eyeground (2). After-effects included fatigue (3) and insomnia (1).
400 ppm (1510 mg/m³)	2	Fatigue and mental confusion (3), headache, muscular weakness, dilated pupils, and pale eyeground (2). After-effects were fatigue (3), skin paresthesia (1), headache (1), and insomnia (2).

TABLE VI-1 (cont.)

Concentration	No. of Exposures	Effects
600 ppm (2660 mg/m³)	1	Extreme fatigue, mental confusion, exhilaration, nausea, headache and dizziness (3), and severe headache (2) after 3 hours of exposure. After 8 hours of exposure, effects included considerable incoordination and staggering gait (3), and several instances of dilated pupils, impaired pupillary light reflex and pale optic discs; after-effects included fatigue and weakness, nausea, nervousness and some confusion (3), severe headache (2), and insomnia (2). Fatigue and nervousness persisted on the following day.
800 ppm (3020 mg/m³)	1	Rapid onset of severe fatigue and, after 3 hours, pronounced nausea, confusion, lack of self-control, and considerable incoordination and staggering gait in all 3 subjects. Also, pupillary light reflex was strongly impaired (1) and optic discs were pale (2). All 3 subjects showed considerable after-effects, lasting at least several days, which included severe nervousness, muscular fatigue and insomnia.

[a] Source: Adapted from von Oettingen et al., 1942a,b

[b] Exposures were twice weekly for 8 weeks. The number of subjects affected is noted in parentheses. Three subjects were used during each exposure.

periods. Pure air was used as a control and tests at any given toluene concentration were repeated only up to 4 times. Results of the study are reported in Table VI-1. Slight effects were reported at 50 and 100 ppm (189.0 and 377.0 mg/m³), but more severe effects were subjectively reported following exposure at 200 ppm (754 mg/m³) toluene. The severity of these effects, (fatigue, confusion and impaired coordination and muscular weakness), increased throughout the exposure levels up to 800 ppm (3020 mg/m³). At 800 ppm, severe fatigue, nausea, confusion, incoordination and lack of muscular control were reported in all three subjects. After-effects, lasting up to several days, included insomnia, nervousness and muscular fatigue.

Two male subjects exposed to toluene vapor (purity not specified) at 200, 400, 600 or 800 ppm for 7-8 hours noted similar effects (Carpenter et al., 1944). The subjective effects included slight exhilaration at 200 ppm; lassitude, nausea and hilarity at 400 ppm; lassitude, boisterousness, verbosity and hilarity at 600 ppm; and extreme lassitude, headaches, slight nausea, verbosity, scotomatous vision, muscular unsteadiness and general intoxication at 800 ppm. Ogata et al. (1970) tested reaction time and perceptual speed among 23 Japanese volunteers exposed to 100 or 200 ppm toluene (377 or 754 mg/m³) for 3 hours or 3 hours followed by a 1-hour break and 4 additional hours of exposure. Exposure to 100 ppm had no effect, while inhalation of 200 ppm resulted in prolonged hand-eye reaction time but had no effect on flicker fusion frequency (the frequency at which stroboscopic light pulses appear as continual illumination).

The effects of acute toluene exposure on perceptual speed and reaction time were determined in 12 male volunteers exposed at 100, 300, 500 or 700 ppm toluene by inhalation through a mouthpiece during four successive 20-minute exposures (Gamberale and Hultengren, 1972). The actual exposure regimen was 100 ppm for 20 minutes, 300 ppm for 20 minutes, 5 minutes of nonexposure, 500 ppm for 20 minutes and 700 ppm for 20 minutes. A series of performance tests for perceptual speed (identical numbers, spokes) and reaction time (simple and choice) was conducted during the final 15 minutes of exposure at each toluene concentration (Table VI-2). The results of Gamberale and Hultengren (1972) indicate that simple reaction time is decreased after exposure to 300 ppm toluene (see Table VI-2). Perceptual speed was not affected significantly until exposure was at the 700 ppm toluene level. Toluene exposure at 100 ppm for 20 minutes appeared to be a NOEL.

Following accidental or industrial exposure to high concentrations of toluene, workers reported exhilaration, light-headedness, clumsiness, dizziness and finally, collapse and unconsciousness. Longley et al. (1967) reported that 26 workmen were exposed to toluene at levels ranging from 10,000-30,000 ppm (conjectural estimate) in the holds of cargo ships. CNS effects progressed in the sequence previously noted. Loss of consciousness occurred within minutes in some cases. No deaths occurred and recovery with no after-effects was quite rapid. The progression of CNS effects begins with an excitatory phase (with euphoria, drunkenness, dizziness and delusions) followed by depression (headache, confusion, incoordination, drowsiness and depressed reflexes). If toluene levels are high enough and exposure duration is sufficiently long, unconsciousness, possibly with

TABLE VI-2

Effect of Toluene Exposure on the Performance of Perceptual Speed and Reaction Time Tests[a,b]

Performance Test	Concentration (ppm)	Mean Test Scores		t-Value
		Experimental Conditions	Control (Air) Conditions	
Identical Numbers[c] (minutes)	100	5.62	5.53	+0.50
	300	5.25	5.29	-0.39
	500	5.13	5.04	+1.34
	700	5.19	4.80	+2.65 ($p<0.05$)
Spokes[d] (seconds)	100	0.5	0.8	-0.08
	300	6.7	3.7	+1.18
	500	3.6	0.2	+1.28
	700	5.4	6.9	+2.51 ($p<0.05$)
Reaction Time - Simple[e] (meters/second)	100	28	30	-0.31
	300	36	22	+2.35 ($p<0.05$)
	500	46	19	+3.88 ($p<0.01$)
	700	53	14	+4.81 ($p<0.01$)

TABLE VI-2 (cont.)

Performance Test	Concentration (ppm)	Mean Test Scores		
		Experimental Conditions	Control (Air) Conditions	t-Value
Reaction Time - Choice[f] (meters/second)	100	25	22	+0.34
	300	29	16	+1.99
	500	32	00	+2.91 ($p<0.05$)
	700	42	08	+3.59 ($p<0.01$)

[a] Source: Adapted from Gamberale and Hultengren, 1972

[b] 12 male subjects were exposed to toluene concentrations of 100, 300, 500 and 700 ppm during four successive 20-minute periods. The tests were performed at each concentration sequentially in the order listed. The number of times each test sequence was repeated was not stated.

[c] Perceptual speed: Identical Numbers. Subjects were instructed to underline the 3-digit number, from a total of 60 columns, that was identical to the number at the head of each column. Performance was measured as the time taken to complete the test.

[d] Perceptual speed: Spokes. Subjects were instructed to connect circles located at random on four pages and numbered from 1-20 in the correct numerical order using a pen. Performance was measured as the mean time taken for the four assignments.

[e] Simple Reaction Time. Subjects were instructed to respond to a signal from a lamp by pressing a push-button. Stimuli were administered at intervals of approximately 10 seconds, an acoustic warning signal was given 3 seconds prior to onset of stimuli, and 30 stimuli were given in each trial Performance was measured as the mean reaction time for the last 20 stimuli administered.

[f] Choice Reaction Time: Stimulus/reply test as above, but there were three pushbuttons equipped with matching stimulus lamps. Stimulus administration followed a random sequence with the number of light signals evenly distributed among the lamps, but the trial and performance measurements were otherwise the same as for simple reaction time.

Degrees of freedom = 11

convulsions, will occur (Helliwell and Murphy, 1979). This progression, with some variation between individuals, is noted in glue sniffers and occupationally exposed workers.

Acute exposure to toluene has been associated with lung and respiratory tract irritation, as well as conjunctival irritation. Two male subjects exposed to toluene vapor at 200 ppm (754 mg/m^3) for 7-8 hours reported mild throat and eye irritation (Carpenter et al., 1944).

Ogata et al. (1970) reported a decrease in pulse rate among 23 volunteers who were exposed to 200 ppm (754 mg/m^3) toluene. Exposure was for 3 hours or for 3 hours followed by a 1-hour break and 4 subsequent hours of exposure. No effect on pulse rate was apparent after exposure at 100 ppm (377 mg/m^3) toluene. Blood pressure of the volunteers was not affected at either level. Astrand et al. (1972) reported that exposure to toluene at 100 or 200 ppm (377 or 754 mg/m^3) for 30 minutes had no effect on pulse rate or electrocardiograms of 15 volunteers. Heart rate and blood pressure were not affected in volunteers exposed to 100-700 ppm (377-2640 mg/m^3) toluene for 20 minutes (Gamberale and Hultengren, 1972) or 50-800 ppm (189-3020 mg/m^3) for 8 hours (von Oettingen et al., 1942a,b).

At least 125 cases of death from solvent sniffing have been reported (Winek et al., 1968; Chiba, 1969; Bass, 1970; Alha et al., 1973; Nomiyama and Nomiyama, 1978). An autopsy of an adolescent boy who died as a result of model airplane glue sniffing showed extreme congestion of the lungs with hemorrhage in the larynx and upper trachea (Winek et al., 1968). Congestion and increased density of the spleen and congestion and darkened coloration of the liver also were noted. All other organs examined were unremarkable. In 19 cases of death among Japanese exposed to toluene-based thinner, Chiba (1969) reported congestion of various organs, pulmonary edema and petechia, and swelling of the brain. Bass (1970) and Alha et al. (1973) reported at least 122 cases of sudden death from solvent sniffing. Most of the sudden deaths were attributed to severe cardiac arrhythmia, which was caused by trichloroethane or fluorinated aerosol propellants. About 10 deaths were attributed solely to toluene.

Subchronic and Chronic Exposure

In a report concerned with occupational exposure to toluene and other solvents, Francone and Braier (1954) reported that in one clinic in Argentina, chronic leukemia patients were given various benzene homologs in an attempt to control the disease. It was reported that toluene was given to leukemics orally in olive oil by capsule, at doses of 10 g/day for a total of 130 g in 3 weeks. The toluene treatment was tolerated without complaints or evidence of clinical side effects. The number of patients treated with toluene could not be ascertained from this publication. The lesser toxicity of toluene compared with benzene was due to the methyl group substituent.

Based on a survey of the symptoms of 100 workers exposed to commercial toluene (formulation not explicated), Wilson (1943) related the level of toluene exposure to patients' symptoms. Workers (type of industry was not reported) were exposed to 50-1500 ppm (189-5660 mg/m^3) toluene for periods of 1-3 weeks. Workers studied were those who had symptoms severe enough to seek medical examination at a hospital. Exposure levels within the work area of each worker were determined soon after they arrived at the hospital for examination. Approximately 60% of the workers were exposed to 50-200 ppm toluene and complained of headache, lassitude and loss of appetite. Wilson (1943) noted that these symptoms were mild and may have been psychogenic. Approximately 30% of the workers reported headache, nausea, anorexia, lassitude and slight impairment of coordination reaction time and temporary memory loss. This group of workers was found to be exposed to 200-500 ppm (754-1885 mg/m³) toluene. In ~10% of the workers, a definite loss of coordination and reduced reaction time were evident. Symptoms of these workers also included headache, nausea, dizziness, loss of appetite and extreme weakness. These workers were exposed to higher toluene (benzene may have been a contaminant) levels, between 500-1500 ppm (1885-5660 mg/m³). Upon examination, most of these workers subchronically exposed to toluene at >500 ppm had reduced red blood cell counts ($\sim 2.5 \times 10^6/m^3$). Two cases also had decreased leukocytes, platelets, polymorphonuclear cells and reticulocytes and increased monocytes. Aplastic anemia was diagnosed in both workers after bone marrow biopsies showed partial degeneration of the hematopoietic tissue. No histological effects were apparent in workers exposed at lower toluene levels.

Effects on the nervous system have been reported in many studies on chronically exposed humans. Munchinger (1964) reported an "organic psychosyndrome" that was characterized by disturbances in memory, thinking and activity, among printers and printers' helpers exposed to toluene levels of ~300 and ~430 ppm (1130 and 1620 mg/m³), respectively. Among a total of 110 workers (the number of printers vs. helpers was not reported), this syndrome was diagnosed in 21 and 40%, respectively, of the printers and the helpers. Results of the Rorschach Test and Knoepfel's 13-Error Test for identifying cognitive disturbances agreed with the diagnosis of the solvent-induced psychosyndrome in 95% of the cases. Lindstrom (1973) determined the psychological and psychomotor performances of 168 male workers who had been exposed to various solvents for 0.1-30 years (exposure levels not reported). Twenty-six of the workers were exposed predominantly to toluene and 25 were exposed to toluene and xylene, while the remaining workers were exposed to other solvents such as trichloroethylene, thinners and tetrachloroethylene. In comparison with a group of 50 unexposed workers, the solvent-exposed group showed poorer performance in tests of sensorimotor speed, psychomotor skills and visual accuracy (Bourdon-Wiersma vigilance test, Santa Ana dexterity test, Mira psychomotor test). Rorschach personality test performance was comparable between the control and exposed groups.

A battery of intelligence and behavioral tests was conducted on a group of 100 automobile painters and 101 age-matched, nonexposed controls (Hanninen et al., 1976). Painters were exposed to toluene at 30.6 ppm and other solvents such as butyl acetate and xylene at lower concentrations (<6.8 ppm) for 1-40 years (average exposure of 14.8 with a standard deviation of 8.5 years). Tests included five memory or learning tasks, four tests of psychomotor performance, three visual tests, one verbal intelligence test and the Rorschach test for personality changes (Table VI-3). This study demonstrated that a reduction of performance was evident in the auto-painters for most tests. Seppalainen et al. (1978) tested the same group of painters for neurophysiological effects. EEGs were conducted on 102 painters and 102 unexposed controls. Abnormal EEG patterns were detected in 32 painters and 37 controls. It also was reported that 26 of the painters admitted having a complex of four subjective symptoms (frequent

TABLE VI-3

Performance Tests: Means, Standard Deviations, and Significances Between Toluene-Exposed Painters and Age-Matched Controls[a]

Test	Significance Means and Standard Deviations		Significance Differences (t-test)
	Exposed (N = 100)	Nonexposed (N = 101)	
WAIS[b] Similarities test[c]	19.4 ± 3.1	2.9 ± 2.1	$p<0.001$
WAIS Picture Completion[d]	14.9 ± 2.9	16.2 ± 2.3	$p<0.001$
WAIS Block Design[e]	34.6 ± 7.[illegible]	39.6 ± 5.6	$p<0.001$
Figure Identification[f]	32.0 ± 9.0	36.7 ± 9.8	$p<0.001$
WAIS and WMS[g] Digit Span[h]	10.6 ± 1.6	11.5 ± 1.8	$p<0.001$
WMS Logical Memory[i]	11.7 ± 3.7	13.9 ± 3.1	$p<0.001$
WMS Associate Learning[j]	15.3 ± 3.6	17.1 ± 2.6	$p<0.001$
Benton Test for Visual Reproduction	21.1 ± 3.1	22.6 ± 2.3	$p<0.001$
Benton Test for Visual Retention	8.2 ± 1.5	8.7 ± 1.3	$p<0.05$
SADT - right hand[k]	44.7 ± 5.7	47.5 ± 5.8	$p<0.01$
SADT - left hand[k]	42.3 ± 5.4	43.6 ± 5.1	NS
SADT - coordination with both hands[k]	29.0 ± 5.4	31.5 ± 5.7	$p<0.01$
Finger Tapping - right hand[l]	02.5 ± 29.2	09.6 ± 23.8	NS
Finger Tapping - left hand[l]	86.7 ± 28.5	96.4 ± 22.4	$p<0.05$
Reaction Time (Simple) - right hand	12.4 ± 2.9	11.9 ± 1.4	NS
Reaction Time (Simple) - left hand	12.1 ± 3.0	11.7 ± 1.4	NS

TABLE VI-3 (cont.)

Test	Significance Means and Standard Deviations		Significance Differences (t-test)
	Exposed (N = 100)	Nonexposed (N = 101)	
Reaction Time (Choice)	9.1 ± 1.8	9.1 ± 1.2	NS
Mira Test[m]	18.8 ± 3.8	20.3 ± 4.6	$p<0.01$[n]
Mira Test[m]	2.2 ± 1.0	2.0 ± 0.8	$p<0.05$

[a] Source: Adapted from Hanninen et al., 1976

[b] Wechsler Adult Intelligence Scale.

[c] Measures verbal intelligence and abstraction.

[d] Measures visual intelligence and observation.

[e] Measures visual intelligence and abstraction.

[f] Measures speed of perception and memory for visual details.

[g] Wechsler Memory Scale.

[h] Measures memory for digits.

[i] Measures verbal memory.

[j] Measures verbal memory and learning.

[k] Santa Ana Dexterity Test; measures psychomotor speed.

[l] Measures motor speed.

[m] Test for psychomotor behavior and psychomotor ability; two variables tested.

[n] Paired t-test.

NS = Not significant

headaches, absent-mindedness, ease of falling asleep while watching television and interrupted sleep), while only 12 controls had this complex of symptoms. Twelve of these 26 painters also were among those with abnormal EEGs. None of the differences between exposed painters and controls were statistically significant (Seppalainen et al., 1978).

Using EEG recordings, Rouskova (1975) reported that 18/20 workers exposed to toluene at >250 ppm (943 mg/m³) and 1,1,1-trichloroethane (concentration not reported) had abnormal responses to light stimulation. Light flashes at the frequency of 1-30/second were applied for 10 seconds with 10 seconds between each flash series. A normal response was one that was synchronous or harmonic with the frequency of the flashes. While 90% of the toluene-exposed workers had abnormal EEGs, only 1/20 unexposed controls had an abnormal EEG response to the photic stimulation.

Several investigators have reported subjective symptoms indicative of cerebral and cerebellar effects in persons who abused toluene or toluene-containing solvents for several years (Table VI-4). Boor and Hurtig (1977) reported that an optician who routinely cleaned eyeglasses and contact lenses with toluene in a small confined room also showed signs of cerebral dysfunction. Signs of cerebral and cerebellar effects included ataxia, tremors, equilibrium disorders, nystagmus, positive Babinski reflex, impairments to speech, hearing and vision, impaired concentration and memory, emotional instability and behavioral changes and psychoses. Severity of symptoms was somewhat dependent on duration and intensity of exposure. Many of these effects were reversible after cessation of toluene exposure. In cases where prolonged exposure has occurred, permanent encephalopathy and

TABLE VI-4

Encephalopathic Effects of Chronic Toluene Abuse

Subject (Age)	Exposure History	Effects and Diagnosis	Reference
Male (33 years)	Regularly sniffed toluene for 14 years. Subject purchased a gallon of pure toluene every 4-6 weeks, and inhaled the toluene on an almost daily basis at frequent intervals throughout the day.	Patient initially examined after 6 years by Grabski; signs included ataxia, intention tremors, pyramidal signs and psychosis, which were concluded to be consistent with cerebellar degeneration. After 8 more years of abuse, Knox and Nelson reexamined the patient and concluded that the syndrome was primarily a diffuse cerebral disorder based on findings of ataxia, tremors, limb incoordination, emotional lability, marked snout reflex, and positive Babinski toe reflex; cerebral atrophy was confirmed by EEG and pneumoencephalography.	Grabski, 1961; Knox and Nelson, 1966
Male (30 years)	10-Year history of toluene abuse.	Recurrent headaches, "inappropriate" speech, brief episodes of memory loss, increased irritability, and exaggerated swings in mood. Unremarkable clinical and neurological exam, but nonspecific EEG changes were found that were regarded as consistent with diffuse encephalopathy.	Satran and Dodson, 1963
Female (19 years)	Almost daily sessions of prolonged paint sniffing for 1-1/2 years. Ingredients not specified but it was indicated that toluene was a common ingredient in all the brands sniffed. Previous 4-year history of multiple drug and solvent abuse.	Ataxia, intention tremors of hands and feet, incoordination, hallucinations. Normal EEG, brain scan, arteriography, and pneumoencephalography. The diagnostic impression was cerebellar dysfunction secondary to some toxic factor in the paint. Objective neurological improvement 5 months after sniffing was discontinued.	Kelly, 1975
Male (25 years)	10-Year history of lacquer thinner (99% toluene) abuse; during the last 5 years he had spent virtually all his waking hours inhaling the vapors (1 gallon used every 2 weeks)	Ataxia, mildly slurred speech, nystagmus, and bilateral Babinski signs. Normal EEG, nuclide brain scan, electromyogram, and nerve conduction studies, but a computerized brain scan showed diffuse widening of the cortical and cerebellar sulci. Subjective improvement in condition following abstinence from exposure, but a neurological exam after 9 months was essentially unchanged.	Boor and Hurtig, 1977

TABLE VI-4 (cont.)

Subject (Age)	Exposure History	Effects and Diagnosis	Reference
Male (59 years)	Optician who frequently but intermittently used 99% toluene in a small unventilated room to clean eyeglasses and contact lenses. Unable to smell toluene because of chronic anosmia. Duration of exposure not stated.	Fatigue and clumsiness of the left side, which got progressively worse. Occasional staggering and mildly slurred speech, disturbed concentration and memory. Normal neurological exam, EEG and brain scans. Daily improvement without specific treatment following cessation of exposure.	Boor and Hurtig, 1977
Male (age not stated)	Habitual inhalation of paint thinner (toluene) on the job. Duration not stated.	Bizzare behavior before admittance to hospital. Admitted in an agitated, violent, nearly catatonic state.	Weisenberger, 1977
Male (27 years)	Sniffed unspecified glues and paint thinners for 10 years. From age 25, toluene was involved 4-5 times/week (200-300 mℓ/week used), and from age 26, he inhaled 4-7 times/day (100 mℓ/day used.	Arm and neck tremors, ataxia, incoordination, and equilibrium disorders. No abnormal psychiatric symptoms. Pneumoencephalographic and angiographical evidence of midbrain and cerebral atrophy. Degeneration of the cerebellum suspected.	Sasa et al., 1978
Male (20 years)	3-Year history of daily aerosol spray paint inhalation. Product contained copper, toluene, and xylene as solvents and isobutane propane and methylene chloride as propellants.	Reduced vision, poor color perception, constricted visual fields, normal optic fundi, impaired papillary response, ataxia and nystagmus. Symptoms slowly subsided following cessation of paint sniffing.	Keane, 1978
Male (25 years)	Sniffed toluene for 4 months, starting while on the job using toluene as a solvent in the rubber processing industry.	Delusions and unpredictable behavior. Largactil prescribed because he was thought to have a schizophrenic illness. Symptoms disappeared and did not recur following termination of sniffing.	Tarsh, 1979
Female (18 years)	Inhaled pure toluene since age 12, regularly since age 16 (2 ℓ/month). Sniffed more heavily than usual during the last 2 months.	Personality changes (apathy, irritability, emotional lability, carelessness), vomiting, difficulty in walking, and slurred speech 1-2 weeks before admission. Gait ataxia, incoordination, dysarthria, downbeat nystagmus, bilateral positive Babinski sign, visual and color sense loss, impaired concentration and abstracting ability upon admission. Symptoms consistent with mainly cerebellar-brain stem involvement and possibly optic neuritis. Symptoms decreased when she did not inhale toluene, and disappeared after 8 months.	Malm and Lying-Tunell, 1980

cerebral or cerebellar atrophy (identified by EEG, angiogram or pneumoencephalogram) are noted (Knox and Nelson, 1966; Boor and Hurtig, 1977; Sasa et al., 1978).

The effects of toluene exposure on peripheral nerves and neuromuscular function have been studied by several investigators (Matsushita et al., 1975; Seppalainen et al., 1978). Fifty-nine of the previously noted automobile painters and 53 age-matched controls were tested for maximum motor nerve conduction velocity (MCV), conduction velocity of the slower motor nerves (SMCV), maximal sensory nerve conduction velocity (SCV) and latency time at motor endplates of upper and lower limbs (Seppalainen et al., 1978). No significant differences in the mean values of these parameters were noted between the painters and controls. Several individual conduction velocities, however, were slower. In comparison with historical control values from Seppalainen's research, 12/59 painters had decreased MCVs, SCVs or prolonged motor endplate latencies, while none of the 53 controls had abnormal values (Seppalainen et al., 1978).

A group of 38 female shoemakers who were exposed to glue containing toluene and some gasoline were tested for peripheral nerve and neuromuscular function (Matsushita et al., 1975). Women were exposed to toluene at 15-200 ppm (mean levels ranged from 60-100 ppm), depending on the time of year, for an average of 3 years and 4 months. In some work areas, gasoline levels ranged from 20-50 ppm. Urinary hippuric acid levels among the shoemakers (3.26 ± 0.82 mg/mℓ) and a group of 16 unexposed control women (0.35 ± 0.24 mg/mℓ) were consistent with toluene exposure. Only the number of women having an abnormal patellar-tendon reflex (14/38) was significantly ($p<0.05$) different from controls (1/16), but the ankle-tendon reflex and the grasping strength of the dominant hand also was regarded as being affected by exposure to toluene. Finger agility, measured by the time necessary to move 25 "bulbs" with glass chopsticks, was significantly decreased in the shoemakers. Descriptions of the test procedures were not reported.

It should be noted that information that attributes pure toluene as the causative agent of peripheral nerve dysfunction are not available. In fact, Boor and Hurtig (1977) reported no sensory or neuromuscular effects in a patient who had permanent cerebral effects from prolonged abuse of 99% pure toluene. Altenkirch et al. (1977) presented data that implicated methyl ethyl ketone as the causative agent of peripheral nerve effects. Neuropathologies were noted in paint thinner-sniffing adolescents within 3-4 months after the mixture of the thinner was made to contain 11% methyl ethyl ketone. The amount of toluene remained about the same (29-30%) and n-hexane content was decreased from the previous mixture (31%) to the new formulation (16%).

Chronic occupational exposure to toluene was, before the mid-1950s, associated with hematological and hematopoietic effects (Ferguson et al., 1933; Greenburg et al., 1942; Wilson, 1943). Recently, benzene has been shown to be the causative agent; toluene is not considered toxic to the blood or bone marrow.

Greenburg et al. (1942) studied hematologic parameters of 61 airplane painters who were exposed to 100-1100 ppm toluene, as well as other solvents, for 2 weeks to 5 years. Mildly decreased erythrocyte levels, increased hemoglobin levels and mean corpuscular hemoglobin concentration, lymphocytosis and macrocytosis were noted among the painters. No changes were seen in abnormal granulocytic leukocyte counts, differential granulocyte counts, reticulated erythrocyte counts, platelet counts, erythrocyte sedimentation rates, hematocrit, coagulation time, erythrocyte fragility or serum bilirubin levels.

Banfer (1961) conducted hematologic examinations on 889 rotogravure printers and printers' helpers who were exposed to toluene-containing inks. A group of 478 nonexposed workers (155 were management workers of the rotogravure plant, while 323 were nonplant employees) were used for comparison. Workers were exposed to the inks, which contained commercial toluene with <0.3% benzene, for at least 3 years. Toluene vapor levels ranged from <200-600 ppm, as determined in a translation of the study by NIOSH (1973). The results revealed no significant differences in the total count of leukocytes, lymphocytes, granulocytes, erythrocytes or hemoglobin levels between toluene exposed workers and the control groups (Table VI-5).

Seventeen workers exposed to toluene at 80-160 ppm (mean of 125 ppm) in a V-belt manufacturing plant showed no differences in hemoglobin levels, erythrocyte counts, leukocyte counts and platelet counts than those in a group of 19 nonexposed workers from the same plant (Capellini and Alessio, 1971). Matsushita et al. (1975) found no differences in hematologic parameters (leukocyte count, hematocrit, hemoglobin content and specific gravity of whole blood) among 38 female shoemakers exposed to an average of 60-100 ppm toluene. Of the shoemakers 13/38 had an increased number of "Mommsen's" toxic granules in the neutrophils, while only 1/16 unexposed women showed increased granule numbers.

TABLE VI-5

Hematologic Examination of 889 Rotogravure Workers[a]

	Printers (N = 889)	Controls, Group 1[b] (N = 155)	Controls, Group 2[c] (N = 323)
Erythrocytes			
counts <4x10^6/mm^3	16 (1.79%)	3 (1.93%)	7 (2.10%)
Leukocytes, total			
counts > 8500/mm^3	78 (8.77%)	11 (7.09%)	26 (8.04%)
counts <5000/mm^3	74 (8.32%)	18 (11.61%)	38 (11.76%)
counts <4500/mm^3	28 (3.15%)	4 (2.58)	12 (3.71%)
counts <4000/mm^3	3 (0.33%)	1 (0.64%)	1 (0.30%)
Lymphocytes			
<35% total leukocytes	25 (2.81%)	3 (4.16%)	4 (1.32%)
total counts <5000/mm^3	89 (100%)	55 (100%)	23 (100%)
Granulocytes			
total counts >2000/mm^3	89 (100%)	55 (100%)	23 (100%)
Hemoglobin			
value <13g/100mℓ	4 (0.45%)	4 (2.58%)	4 (1.23%)

[a] Source: Adapted from Banfer, 1961

[b] Unexposed management workers from the same plant

[c] Unexposed individuals not employed at the plant

Studies conducted on the immunocompetence of workers exposed to toluene (80-230 mg/m³), benzene (11-170 mg/m³) and xylene (120-3000 mg/m³) were conducted by Lange et al. (1973a,b) and Smolik et al. (1973). Thirty-five workers, exposed to the three solvents for 1-21 years, were determined to have lower serum IgG and IgA levels but increased IgM levels compared with unexposed controls (Lange et al., 1973a). Ten of the 35 workers had leukocyte agglutinins for autologous leukocytes and sera from these workers had increased leukoagglutination titers when incubated with the three solvents. These data suggest that allergic blood dyscrasia may occur in the workers. Smolik et al. (1973) reported decreased serum complement levels in 79 workers having a similar exposure to toluene, benzene and xylene. The specific causative solvent(s) were not ascertained.

Abuse and occupational exposure to toluene have been associated with hepatotoxic effects in some studies (Greenburg et al., 1942; Grabski, 1961), while other investigators reported no remarkable effects (Capellini and Alessio, 1971; Suhr, 1975; Trevisan and Chiesura, 1978). Grabski (1961) reported hepatomegaly and impaired liver function in a 6-year abuser of pure toluene. Hepatomegaly was noted in 13/61 airplane painters who were exposed to 100-1100 ppm toluene for 2 weeks to 5 years (Greenburg et al., 1942). Liver enlargement while not tender, was diagnosed by palpation. The incidence of hepatomegaly among painters was 3 times that in a group of 430 unexposed workers. Liver enlargement could not be attributed solely to toluene because of the complex paint formulations and the lack of reported dose-response data (Greenburg et al., 1942). Shiojima et al. (1983) reported significant increases ($p<0.01$) in the levels of SGOT and SGPT among two groups of toluene-exposed female workers compared with a control group of hospital nurses (n=50). Group A consisted of eight women rubber bag workers exposed to toluene-containing glue, and workroom vapor levels (determined by personal dosimeters) were ~69 ppm toluene. Group B, consisting of 18 women exposed to toluene and negligable amounts of methyl ethyl ketone in glues used in rubber boat manufacture, were exposed to ~45 ppm toluene. Although differences in liver function were indicated when compared with the control group, the reported SGOT and SGPT levels (21.7, 19.8 and 15.0, 15.1 for Group A and B, respectively) were within historical range. No other consistent differences in hepatic function were evident between toluene-exposed women and controls (Shiojima et al., 1983).

No effect on liver function, as determined by serum protein and protein electrophoresis, was reported in 17 workers exposed to 80-160 ppm toluene in a V-belt manufacturing plant (Capellini and Alessio, 1971). Hepatic function parameters and hepatomegaly were similar but elevated in a group of 100 gravure printers and a group of 100 nonexposed workers from the same company (Suhr, 1975). Liver enlargment was noted in 22% of the printers who were exposed to 200-400 ppm pure toluene for at least 10 years, and in 20% of controls. Increases in SGOT, SGPT, glutamic dehydrogenase or gamma glutamyl transferase levels were noted in 50% of the printers and 51% of the controls. No effects could be attributed to toluene and the cause for hepatotoxic effects in both groups was not determined (Suhr, 1975). Bilirubin, SGOT, gamma glutamyl transpeptidase, alkaline phosphatase, ornithine-carbamyl transferase, Quick's test and protein content were determined in sera of 47 workers exposed to toluene vapor (Trevisan and Chiesura, 1978). All parameters were normal except the level of gamma glutamyl transpeptidase, which was above the historical norm (28 μg/mℓ) in 34% of the workers.

Similarly, in the cases of an optometrist and a glue sniffer exposed to 99% pure toluene, a series of liver function tests were normal (Boor and Hurtig, 1977).

Renal dysfunction has been reported following occupational exposure to toluene and toluene abuse. At least 159 cases of toluene-abuse have been studied during 1957-1967 with pyuria, hematuria and proteinuria being the most common signs of renal effects (Table VI-6). Christiansson and Karlsson (1957) reported no abnormalities in urine samples from 32 paint thinner sniffers, while Sokol and Robinson (1963) noted that 67 of the 89 glue-sniffers had some type of urinary abnormality (details not reported). O'Brien et al. (1971) reported hematuria, proteinuria, elevated serum creatine and decreased urinary output in a patient who had a 3-year history of toluene abuse. At the time of the study, this patient was admitted to the hospital as a result of a deliberate 6-hour inhalation exposure to cleaning fluid that contained 80% toluene.

In several recent reports (Taher et al., 1974; Fischman and Oster, 1979; Bennett and Forman, 1980; Kroeger et al., 1980; Moss et al., 1980), toluene abuse has been associated with metabolic and distal renal tubular acidosis (Table VI-7). Serious electrolyte imbalances, primarily hypokalemia and hyperchloremia, are caused by a toluene-induced decrease in hydrogen ion secretion in the distal convoluted tubules. Moss et al. (1980) additionally reported increased urinary excretion of glucose, uric acid, phosphate, amino acids and calcium, indicating proximal tubule dysfunction. The cases in which acidosis was diagnosed were those having a history of toluene abuse, but upon treatment and cessation of exposure, normal renal functions were restored. Fischman and Oster (1979) reported a high anion gap metabolic

TABLE VI-6

Renal Function Investigations of Glue Sniffers[a,b]

Number of Patients	Pyuria	Hematuria	Proteinuria	Clearances	Azotemia	Reference
32	All 32 urine samples "normal"; details not given	ND	ND	ND	ND	Christiansson and Karlsson, 1957
27	0	2	0	ND	0	Massengale et al., 1963
89[c]	2	4	2	ND	ND	Sokol and Robinson, 1963
15	0	0	1	PSP[d] 0/13	0/7	Barman et al., 1964
16	6	3	5/13	Urea 1/7	0/9	Press and Done, 1967a,b

[a] Source: Adapted from Press and Done, 1967a,b

[b] Exposures were to toluene-containing plastic cements except in the Christiansson and Karlsson (1957) study, in which the subjects examined had sniffed paint thinner.

[c] Urinary abnormalities were found in 67/89 glue sniffers.

[d] Phenosulfonphthalein clearance in 2 hours.

ND = not determined

TABLE VI-7

Toluene-Induced Metabolic Acidosis

Subject (Age)	Exposure History	Symptoms	Clinical Findings	Reference
Male (23 year)	Sniffed glue and pure toluene intermittently for 6 years.	Several episodes of muscle weakness following prolonged (i.e., 4-7 day) inhalation sessions. One instance of flaccid paralysis.	Hypokalemia with hyperchloremic metabolic acidosis. Elevated urinary pH. Toluene detected in blood.	Taher et al., 1974
Female (20 year)	Two 3- to 5-day episodes of sniffing aerosol paint containing 60% toluene within 4 weeks.	Nausea.	Hyperchloremic acidosis. Elevated urinary pH. Toluene detected in blood.	Taher et al., 1974
Female (17 year)	Sniffed transmission fluid containing 100% toluene for 5 days.^{b}	Persistent vomiting.	High anion gap^a metabolic acidosis.	Fischman and Oster, 1979
Female (21 year)	Intermittently sniffed transmission fluid containing 100% toluene for at least 5 years.^{b}	Hospitalized on six occasions within a 16-month period. Severe weight loss (18 kg) at first admission. Recurrent symptoms of vomiting, muscle weakness, and lethargy. After the 6th episode, patient died of cardiopulmonary arrest.	Hypokalemia. Hyperchloremic metabolic acidosis and high urinary pH on 1st and 6th admissions. High anion gap metabolic acidosis on the other admissions.	Fischman and Oster, 1979
Female (25 year)	Frequent sniffing of transmission fluid containing 100% toluene during a 5-year period.^{b}	Persistent vomiting, lethargy and muscle weakness.	Normal anion gap hyperchloremic metabolic acidosis with severe hypokalemia.	Fischman and Oster, 1979
Male (23 year)	Sniffed toluene on a "regular" basis for 5 years. Form not specified.	Hospitalized 4 times within 15 months. History of vomiting, flank pain, and paralysis of the lower extremities.	Recurrent uretal and renal calculi (4 stones total). Hyperchloremic metabolic acidosis and hypokalemia. Acidic urine.	Kroeger et al., 1980
Female (27 year)	Daily inhalation of glue for 9 months.	Lethargy, weakness and ataxia. Microscopic hematuria and sterile pyuria.	Hyperchloremic metabolic acidosis, hypokalemia, hypocalcemia, hypophosphatemia and hypouricemia. Increased excretion of 14 amino acids and glucose.	Moss et al., 1980
Four individuals (ages and sexes not stated)	Glue or paint sniffers (details not stated).	Not stated.	Hyperchloremic metabolic acidosis with hypobicarbonatemia.	Moss et al., 1980
Male (22 year)	Abused a lacquer thinner (99% toluene) for 8 years.	Abdominal pain, vomiting, generalized weakness and diminished reflexes.	Hypokalemic and hypochloremic metabolic acidosis.	Bennett and Forman, 1980

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[a] Anion gap is defined as serum Na - (Cl + HCO_3) in milliequivalents per liter.

[b] Toluene is not ordinarily a component of transmission fluid (Fischman and Oster, 1979).

acidosis with hypokalemia in two abusers of 100% toluene. Renal impairment with ketonemia and elevated lactate and benzoic and hippuric acid metabolites of toluene were suggested as the cause of this acidosis.

Urinalysis of 91 specimens from the 61 airplane painters (donor number not specified) exposed to toluene vapor at 100-1100 ppm (377-4150 mg/m^3) for 2 weeks to 5 years included determinations of albumin, sugar, formed elements and specific gravity. All parameters were normal (Greenburg et al., 1942). Matsushita et al. (1975) reported no abnormalities after urinalysis for sugar, protein, bilirubin, urobilinogen, occult blood and ketone bodies, among samples from 38 female shoemakers. Only hippuric acid excretion was elevated in these women, consistent with their exposure to toluene at 60-100 ppm for an average duration of 3 years and 4 months.

The National Research Council (NRC, 1980) cautioned that some of the reported CNS effects may actually be attributable to altered renal function. For example, hypokalemia can cause muscular weakness and flaccid paralysis. Some of the symptoms noted in Table VI-7, such as lethargy, ataxia, muscular weakness, paralysis and nausea, may be manifestations of metabolic and renal effects.

Reproductive Effects. Toutant and Lippmann (1979) reported a case of a child born with microcephaly, hypoplastic mandible, pronounced sacral dimple, sloping forehead, flat nasal bridge and uncoordinated arm movements. The mother of this child had a 14-year history of major addiction to solvents (primarily toluene) as well as an increased alcohol intake. The symptoms of the child were described as fetal alcohol syndrome and were not attributed to the mother's solvent abuse.

In a matched-control study, Holmberg (1979) attempted to identify those mothers who had exposure to noxious substances and had given birth to children with congenital CNS defects. Based on Finnish maternity records, the matched-control mother was the one whose delivery immediately preceded that of the case mother. Of the 120 case mothers, 14 reported having exposure to organic solvents during the first trimester of pregnancy, while 3/120 control mothers reported such exposure. Only two of the case mothers were exposed to toluene. One who worked in the metal manufacturing industry gave birth to a child with hydrocephaly, agenesis of the corpus callosum and pulmonary hyperplasia. The other mother, age 42, gave birth to a child with hydroencephaly and was exposed to many solvents in the rubber industry. This mother had previously borne a child with brain injury.

Matsushita et al. (1975) reported that 19/38 female shoemakers, exposed to mean toluene levels of 60-100 ppm for an average of 3 years, 4 months duration, complained of dysmenorrhea. Shoemakers were also exposed to gasoline at 20-50 ppm. A questionnaire was used to identify dysmenorrhea. In a group of 16 controls, only 3 reported this abnormality.

Based on an abstract of a Polish study (Michon, 1965), disturbances in menstruation, including prolonged and more intense bleeding, were reported in a group of 500 shoe factory empolyees exposed to benzene (<100 mg/m^3), xylene (<250 mg/m^3) and toluene (<250 mg/m^3). An abstracted Russian study (Syrovadko, 1977) indicated that female workers exposed to 25-350 mg/m^3 toluene and other solvents (from electrical organosiliceous varnishes) had menstrual disorders. Neonates of these women were reported often to be underweight, have frequent fetal asphyxia, and show "belated" nursing activity.

Toluene was not determined to be the direct causative agent in any of these reproductive effects.

High Risk Subpopulations

Based on a historical perspective, the deliberate abusers of toluene and toluene-containing glues or solvent mixtures appear to be those most likely to succumb to the toxic effects of toluene. This group inhales high concentrations of toluene vapors for their intoxicating and euphoric effects. This practice is often repeated daily. Because of psychological dependence and a developed tolerance of toluene vapors, exposure often is continued chronically at increasing exposure levels and for longer durations. Toluene concentrations inhaled by glue sniffers may reach saturation, which is near 30,000 ppm (113,100 mg/m^3).

Millions of workers may be exposed to toluene by inhalation from the work-place air. Toluene's ubiquitous nature as a solvent leads to its use in many occupational settings. Workers may be exposed to toluene during its production or processing by petroleum refinement or from styrene-production and coke-oven byproducts. The major industries using refined toluene are paint, paint thinner and coatings operations. A significant amount of toluene is also used in adhesives, inks and other solvent applications. As a raw material, toluene is used in the production of toluene diisocyanate, benzylchloride, benzoic acid, xylene and vinyl toluene. As a result of the broad spectrum of industrial uses of toluene, many workers are exposed and may be at higher risk of developing toxic symptoms.

Occupationally exposed groups that have experienced toxic symptoms attributed to toluene includes workers from the shoemaking industry (Matsushita et al., 1975), automobile painters (Hanninen et al., 1976; Seppalainen et al., 1978), airplane painters (Greenburg et al., 1942) and printers and printers' helpers (Banfer, 1961; Munchinger, 1964). Workers in these industries were obviously at high risk. Workers in many other industries and occupations may be at equally high risk.

Cigarette smokers are also exposed to toluene at significant levels. Several reports (Dalhamn et al., 1968; Holzer et al., 1976; NRC, 1980) indicate that each cigarette contains ~1.1 mg toluene and the smoker absorbs at least 0.1 mg toluene/cigarette. Assuming 0.1 mg toluene/cigarette, a heavy smoker (60 cigarettes/day) may absorb a considerable amount of toluene over a chronic period. Smoking workers would therefore be at greatest risk, since they would be exposed to toluene from cigarettes, occupational use and various sources of toluene (in drinking water and air).

Summary

Acute toxicity studies of toluene in humans have dealt with experimental exposure, intentional abuse exposure (e.g., glue sniffing) and accidental occupational exposure. Toluene exposure has almost exclusively been by inhalation. Of foremost concern are the effects of toluene on the CNS following acute exposure. Toluene at ~200 ppm (754 mg/m^3) for 8 hours of

exposure has caused symptoms indicating CNS toxicity (fatigue, headache, nausea, muscular weakness, confusion and incoordination) (von Oettingen et al., 1942a,b; Carpenter et al., 1944). Reaction time was increased at 200-300 ppm toluene (Ogata et al., 1970; Gamberale and Hultengren, 1972). Toluene vapor at 100 ppm for 8-hour exposures appeared to be the NOEL. Exposure to high levels of toluene vapor, either by abuse or industrial accidents, first results in CNS stimulation (with dizziness and exhilaration), followed by CNS depression (with eventual loss of consciousness and death) (Longley et al., 1967; Winek et al., 1968; Helliwell and Murphy, 1979). Deaths from acute toluene exposure appear to be caused by respiratory congestion and failure and possibly by cardiac arrhythmia.

Subchronic occupational exposure to toluene (for 1-3 weeks) at levels of 50-1500 ppm (189-5660 mg/m^3) resulted in symptoms similar to those seen in acute exposure studies (Wilson, 1943). At levels of 50-200 ppm, workers complained of headache, lassitude and loss of appetite. More severe symptoms, including headache, nausea, anorexia, lassitude, incoordination, decreased reaction time and temporary memory loss, were reported by workers exposed at 200-500 ppm (754-1885 mg/m^3). The severity of these same symptoms increased in workers exposed at 500-1500 ppm toluene (Wilson, 1943).

Chronic toluene exposure, either occupationally or by deliberate abuse, has been associated primarily with CNS and possibly peripheral nervous system effects. Disturbances in memory, thinking, psychomotor skills, visual accuracy and sensorimotor speed were reported in occupationally exposed humans (Munchinger, 1964; Hanninen et al., 1976). Hanninen et al. (1976)

reported many differences in performance test results between nonexposed workers and painters exposed to ~30.6 ppm toluene for an average of 14.8 years (see Table VI-3). Effects indicative of cerebral and cerebellar dysfunction, such as ataxia, tremors, equilibrium disorders, impaired speech, vision and hearing and impaired memory and coordination, have been reported in chronic abusers of toluene (Knox and Nelson, 1966; Boor and Hurtig, 1977; Sasa et al., 1978). Although several studies have suggested decreased peripheral nerve function in toluene exposed workers (Seppalainen et al., 1978; Matsushita et al., 1975), no studies implicating pure toluene as the causative agent were available.

Hematological and hematopoietic effects of toluene noted in studies before 1955 (Ferguson et al., 1933; Greenburg et al., 1942; Wilson, 1943) have been attributed to the benzene contaminant (which accounted for up to 20% in some samples of toluene). Recent studies have detected no hematological effect of toluene (Banfer, 1961; Capellini and Alessio, 1971; Matsushita et al., 1975). Hepatomegaly and hepatic function changes have been reported in some toluene-exposed groups (Greenburg et al., 1942; Grabski, 1961), while no hepatic effects were reported in other groups (Capellini and Alessio, 1971; Suhr, 1975; Trevisan and Chiesura, 1978). Renal function appears to be affected by chronic toluene exposure. Metabolic and distal renal tubular acidosis and electrolyte imbalances have been reported in chronic toluene abusers. Apparently, toluene inhibits the hydrogen ion secretion in the distal convoluted tubules. No renal effects were reported in occupationally exposed workers (Greenburg et al., 1942; Matsushita et al., 1975).

The potential number of workers exposed to varying levels of toluene is great. Those workers exposed chronically to toluene during its production, in solvents and in glues, are probably at increased risk. Abusers of toluene are at highest risk of the undesirable toxic effects of toluene, since they are chronically exposed to high levels of the solvent.

VII. MECHANISMS OF TOXICITY

Following absorption, toluene is apparently transformed into less toxic substances, including benzyl alcohol, benzoic acid and hippuric acid, and is rapidly excreted (see Chapter 3). Unchanged toluene may be eliminated in the expired air at nearly 18-20% of the absorbed dose in rabbits and humans (Smith et al., 1954; Nomiyama and Nomiyama, 1974b). Since most toluene exposure is by inhalation and much of the absorbed toluene is excreted, unchanged, through the lungs, the pulmonary epithelium could be the primary site of tissue-toluene interaction. Gerarde (1959) reported that alveolar and capillary epithelial irritation by alkylbenzenes, such as toluene, leads to permeability changes within the lung. This can lead to petechial and gross hemorrhage and diapedesis. von Oettingen et al. (1942a) noted slight and severe pulmonary irritation and lesions in rats exposed by inhalation to 200 or 600, and 2500 or 5000 ppm toluene, respectively (see Table V-2). Fabre et al. (1955) reported pulmonary congestion in dogs exposed to 2000 ppm toluene by inhalation (6 hours/day, 6 days/week for 4 months, then 2660 ppm for 8 hours/day, 6 days/week for 2 months). The autopsy of an adolescent who died as a result of toluene exposure from model airplane glue revealed extreme frothiness and congestion in the lungs (Winek et al., 1968). Decreased crepitation throughout the lungs and petechial hemorrhage in the larynx and trachea were also reported.

Effects on the CNS are of greatest concern following exposure to toluene. The effects of toluene exposure are probably first evident in the CNS for three reasons. Being lipophilic, toluene has an affinity for nerve tissue and myelin because of their high lipid content. Secondly, the sensitive nature of nerve physiology makes the nervous system the most susceptible tissue to the structurally nonspecific effect of toluene (Gerarde, 1959). Finally, the rapid circulation of blood to the brain also contributes to the early onset of CNS effects. The toxic action of toluene and other alkylbenzenes is dependent on the lipophilicity of the solvent and on the number of molecules present in a cell ("physical toxicity") and not on a specific type of molecule interacting with a specific target organelle or cell component (Gerarde, 1959). The nerve cell would be expected to be sensitive to lower levels of toxicant than other cell types. Impairment of axonal transport and action potential propagation would result in CNS symptoms and peripheral nerve effects.

Renal effects attributed to toluene exposure also have been reported (see Table VI-7). Acidosis and electrolyte abnormalities have been related primarily to toluene's impairment of hydrogen ion secretion in the distal convoluted tubules. Impairment of hydrogen ion secretion may be due to tubular epithelium permeability changes, similar to those assumed to occur in the lungs (Gerarde, 1959). Permeability changes in both the lungs and kidneys may be caused by an interaction between structural lipids and toluene. Moss et al. (1980) reported pathological levels of urinary amino acids, glucose, phosphate, calcium and uric acid, consistent with permeability changes in renal epithelium. The NRC (1980) has suggested that altered renal filtration, causing electrolyte imbalances and acidosis, may lead to some adverse effects, such as lethargy and muscular weakness, associated with chronic toluene exposure. Hypokalemia, for example, can cause muscular weakness and flaccid paralysis.

Enzyme Induction

The effects of toluene exposure on hepatic cytochrome P-450 concentration and activity were determined in male Sprague-Dawley rats treated at 500, 1500 or 3000 ppm 6 hours/day for 3 consecutive days (Toftgaard and Nilsen, 1981). Rats were fasted for 24 hours prior to the preparation of liver microsomes. The concentration of cytochrome P-450 was significantly ($p<0.05$) increased in a dose-related manner in rats exposed to 1500 and 3000 ppm toluene. The formation of all metabolites of biphenyl and benzo[a]-pyrene (used to identify increases in specific pathways) also was increased, indicating a general, nonselective increase in cytochrome P-450 (Toftgaard and Nilsen, 1981; Toftgaard et al., 1982). Toftgaard et al. (1982) noted that toluene exposure increased the metabolism of benzo[a]pyrene to benzo-[a]pyrene-4,5-dihydrodiol to the greatest extent. It was suggested that the precursor of this metabolite, the 4,5-epoxide of benzo[a]pyrene, is much less potent as a mutagen and carcinogen than is 7,8-epoxide-benzo[a]pyrene.

Brain and liver tissues from toluene-treated rats were used to study the effect on cytochrome P-450 dependent N-demethylation and glutathione-S-transferase (Chand and Clausen, 1982). Female Wistar rats were given i.p. injections for 21 days with 0.87 g toluene/kg in corn oil and fasted along with controls for 20 hours before sacrifice. N-demethylation of p-chloro-N-methylaniline was increased 33.8% in liver microsomes from toluene-treated rats compared with controls. Similarly, glutathione-S-transferase activity was increased 17.1%. In the brain tissue microsomes, N-demethylation was increased only 3.5%, while glutathione-S-transferase activity was increased 10.3%.

Synergistic/Antagonistic Effects

Toluene and benzene may be metabolized by similar hepatic enzyme systems (Mungikar and Pawar, 1976; Pawar et al., 1976). In these studies, hepatic aminopyrine N-demethylase, NADPH-linked peroxidation enzyme activities were reduced, while acetanilide hydroxylase was increased in both toluene- or benzene-pretreated rats. Pretreatment with phenobarbital, however, attenuated the narcotic effect of toluene (i.p. injected) on female rats and male mice, and accelerated the excretion of urinary hippuric acid (Ikeda and Ohtsuji, 1971; Koga and Ohmiya, 1978). Andrews et al. (1977) reported that toluene, in an *in vitro* hepatic microsome preparation, was a competitive inhibitor of benzene metabolism. Ikeda et al. (1972) noted that each, when co-administered with the other, could reduce the excretion of metabolites of the other, indicating an equal inhibition. Toluene inhibition of benzene metabolism was dose-dependent and was not evident in rats treated with relatively low doses of benzene of 24.2-97.7 mg/kg and equimolar toluene (Sato and Nakajima, 1979b); however, benzene at 390.6 mg/kg was metabolized more slowly when simultaneously administered with equimolar toluene.

The simultaneous administration (s.c.) of toluene (184 mg/kg) and benzene (156 mg/kg; ^{14}C) caused a significant ($p<0.05$) delay in the excretion of urinary phenol (^{14}C), a benzene metabolite, for up to 48 hours in male Wistar rats (Gut et al., 1981). The metabolite profile, however, was not altered significantly. When radiolabeled toluene (^{14}C) was co-administered with benzene (same doses as before by s.c. injection), the elimination rate of toluene metabolites was not affected notably. Gut et al. (1981) also reported that the elimination of thiocyanate from orally administered acrylonitrile (26.5 mg/kg) was reduced by simultaneous administration of toluene (46 mg/kg). Mortality in mice was 0/24 or 7/8, 5/8, 12/16 and 7/8 when 60 mg/kg acrylonitrile was administered i.p. or when co-administered with toluene (s.c.) at 104, 208, 312 and 416 mg/kg (non-lethal doses), respectively. These data suggest that the increase in acrylonitrile toxicity is caused by toluene. No data were presented on the increased lethality of toluene caused by acrylonitrile (Gut et al., 1981).

In humans, benzene (25 ppm) and toluene (100 ppm) administered simultaneously had no effect on the elimination of either compound from the body (Sato and Nakajima, 1979b). Elimination rates from the blood and alveolar air for the two compounds were virtually the same as each administered separately. Sato and Nakajima (1979b) noted that the absorption, distribution, metabolism and excretion of either toluene or benzene were not affected when the compounds were co-administered at their respective TLV (100 ppm for toluene; 25 ppm for benzene).

In male rats injected (i.p.) with 0.1 or 0.2 mg/kg toluene and m-xylene (equal doses), the total urinary excretion of hippuric acid and m-methylhippuric acid was not different from amounts excreted by singly-treated rats. The rate of urinary excretion of the metabolites was decreased slightly (Ogata and Fujii, 1979). Suzuki et al. (1974) reported that simultaneous i.p. administration of toluene (1.18 g/kg) and n-hexane (0.91 g/kg) did not affect the urinary excretion of hippuric acid in female rats. Co-administration of toluene and n-hexane reduced the excretion of n-hexane metabolites but did not appear to affect the metabolism of toluene to hippuric acid and o-cresol (Perbellini et al., 1982). Simultaneous exposure to toluene and trichloroethylene (430 and 730 mg/kg i.p. injections, respectively) reduced the amounts of urinary metabolites of both compounds compared with those recovered after administration of either solvent alone (Ikeda, 1974). Withey and Hall (1975) noted that mixtures of toluene and trichloroethylene administered by intubation in rats did not represent an additive model of metabolism. Perchloroethylene administered in conjunction with toluene by the oral route in rats appeared to potentiate the toxicity of toluene (Smyth et al., 1969b).

Administration of ethanol (orally) and toluene (inhalation; 1060 ppm 6 hours/day, 5 days/week) simultaneously to rats for 4 weeks had no effect on hematocrit, electrocardiogram or heart histology or histochemistry (Morvai and Ungvary, 1979). Toluene was reported to increase vascular resistance of the myocardium and to decrease blood flow to the cerebrum. Alcohol reduced blood pressure, cardiac index and blood flow throughout the body. Vascular resistance of the myocardium was increased by alcohol ingestion. Morvai and Ungvary (1979) noted that an additive effect on increased vascular resistance of the myocardium was produced by the combined exposure to the two solvents. Hudak et al. (1978) reported that hepatic microsomal and mitochondrial changes are potentiated by simultaneous exposure to ethanol and toluene in rats. _In vitro_, ethanol administered ~16-18 hours before toluene caused a 2-fold increase in toluene metabolism. The 16- to 18-hour lag time for this enhancement was necessary for the induction of MFO enzymes by ethanol.

In humans, however, toluene metabolism was inhibited by large doses of ethanol (Waldron et al., 1983). In volunteers (n=7) exposed to 80 ppm (302 mg/m^3) toluene, blood levels of toluene were ~42.5% higher with exposure to ethanol than during toluene exposure alone. Subjects received vodka orally at 1.5 mℓ/kg (~500 mg ethanol/kg) after 3 hours of exposure to toluene. Waldron et al. (1983) also noted that occupationally-exposed men who drank alcohol regularly had lower toluene-blood levels than those who were nondrinkers. Waldron et al. (1983) suggested that competition exists between toluene and ethanol for alcohol dehydrogenase and that regular alcohol consumption can induce the oxidizing enzymes.

Toluene co-administered with acetylsalicyclic acid (aspirin) to pregnant rats was found to increase the maternal and embryonic toxicity of aspirin. CFY rats were treated with toluene by inhalation at 1000, 2000 or 3600 mg/m^3 continuously on days 10-13 of gestation and given 250 mg aspirin by gavage on day 12 (Ungvary et al., 1983). Toluene augmented the decreased weight gain and increased relative liver weight in dams treated with 250 mg aspirin (effects were similar to those seen in dams treated at 500 mg aspirin alone). In fetuses examined on day 21 of development, toluene increased ($p<0.01$) the frequency of skeletal abnormalities (missing thoracic vertebrae, shortened axillary bones, extra 14th ribs) and at high doses, increased internal and external malformations (renal abnormalities, clubfoot, cleft palate, polydactyly) (Ungvary et al., 1983).

Summary

Although pulmonary and renal effects have been reported in toluene-exposed animals and humans, the primary mechanism of toluene toxicity is through its effects on nervous tissue. Pulmonary and renal effects apparently are due to changes in epithelial permeability, possibly through toluene's interaction with cellular membrane (structural) lipids. The effects of toluene exposure, whether acute or chronic, are seen first on the CNS. Toluene acts as a physical toxicant (Gerarde, 1959). Therefore, lower toluene exposure levels are likely to impair CNS function and tissue more than any other type of nervous tissue.

It was suggested that toluene and benzene are metabolized by similar enzymatic pathways in the liver, and toluene may be equally or preferentially oxidized when exposure is to both compounds. Toluene also is metabolized preferentially when exposure includes acrylonitrile (Gut et al., 1981). Toluene may potentiate acrylonitrile toxicity, but toluene toxicity would not be expected to be increased. Simultaneous exposure to toluene and m-xylene or n-hexane did not appear to affect greatly the rate of toluene metabolism in rats. Morvai and Ungvary (1979) suggested that both toluene and ethanol caused increased vascular resistance, and that simultaneous exposure to both compounds may result in an additive effect. Ethanol exposure was reported to inhibit toluene metabolism in human volunteers, possibly increasing toluene toxicity (Waldron et al., 1983). Toluene potentiated the maternal and embryotoxic effects of aspirin when both were administered to rats (Ungvary et al., 1983).

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Introduction

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{[Uncertainty Factor(s) x Modifying Factor]}} = \underline{\quad\quad} \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicological effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the U.S. EPA (1986a) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]
- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]
- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]
- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{(\text{RfD}) \times (\text{Body weight in kg})}{\text{Drinking Water Volume in } \ell/\text{day}} = \underline{\quad\quad} \text{ mg}/\ell$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 ℓ/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{(\text{NOAEL or LOAEL}) \times (\text{bw})}{(\text{UF}) \times (\underline{\quad\quad} \ \ell/\text{day})} = \underline{\quad\quad} \text{ mg}/\ell$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 ℓ water per day.
2. 10-day HA for a 10 kg child ingesting 1 ℓ water per day.
3. Longer-term HA for a 10 kg child ingesting 1 ℓ water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 ℓ water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
>
> Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
>
> Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
>
> Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
>
> Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 ℓ of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects

Data on the effects of acute toluene exposure by the oral route in animals or humans are minimal. The oral LD_{50} in rats ranges from 5.5-7.5 g/kg (see Table V-1).

The effects of toluene following acute inhalation exposure have been examined in rats, mice, rabbits, guinea pigs and dogs (see Table V-1). Bonnet et al. (1982) reported that the LC_{50} for toluene (99.5% purity) inhalation for 6 hours was 4618 ppm (17,400 mg/m^3) and 6949 ppm (26,200 mg/m^3) in rats and mice, respectively. In earlier studies (Cameron et al., 1938), mice also were less sensitive to toluene than were rats, having 10 and 60% mortality, respectively, following exposure at 24,400 ppm (92,000 mg/m^3) for 1.5 hours. Exposure at 1600 (6030 mg/m^3) toluene for 18-20 hours in rats caused muscular twitching and eventual death (Batchelor, 1927). No notable effects were reported in rats exposed at 620 ppm (2340 mg/m³) toluene for 18-20 hours (Batchelor, 1927).

In humans acutely exposed to toluene by inhalation, either experimentally or by glue sniffing, the first effects observed are indicative of CNS dysfunction. von Oettingen et al. (1942a,b) exposed volunteers to 0, 50, 100, 200, 300, 400, 600 or 800 ppm (0, 189, 377, 754, 1130, 1510, 2260 or 3020 mg/m³) for 8 hours and reported a progression of effects with increasing dose. As noted in Table VI-1, slight effects such as sleepiness and headache (not consistently reported and possibly psychogenic) were noted by subjects exposed at 50 and 100 ppm toluene. More severe effects attributable to toluene exposure, such as fatigue, confusion, muscular weakness and incoordination, were reported more consistently at 200 ppm. Based upon exposure for 3 hours followed by 1 hour of nonexposure and 4 subsequent exposure hours, Ogata et al. (1970) noted that toluene at 100 ppm had no effect on perceptual speed or reaction time in Japanese volunteers. Toluene at 200 ppm, however, caused a decrease in hand-eye reaction time and pulse rate among the 23 volunteers. Studies by Carpenter et al. (1944) and Gamberale and Hultengren (1972) also indicate that 100 ppm is near the acute NOEL for humans.

Short-term and subchronic testing of toluene by the oral route is limited to a 6-month study by Wolf et al. (1956) in female Wistar-derived rats and a 3-week study by Francone and Braier (1954) in humans. In the former study, three groups of 10 female rats each were given toluene (94.4% purity) in olive oil at 118, 354 or 590 mg/kg/bw by stomach tube. Toluene was administered 138 times during the 193-day study. A group of 20 olive oil-treated rats served as controls. Hematological, behavioral, gross and histopathological examinations were conducted to determine a toxic effect. Only the liver and kidney were examined histologically. No toxic effects were reported in rats at any dose level of toluene; therefore, the authors suggested that the NOEL was >590 mg/kg/day when treatment was on 5 days/week during the 193-day study. In human leukemia patients, 10 g toluene orally/day for a total dose of 130 g over a 3-week period (~90 mg/kg/day) was tolerated with no apparent effects (Francone and Braier, 1954).

Inhalation of toluene at 200 ppm (754 mg/m^3) 7 hours/day, 5 days/week for 6 weeks resulted in evidence of slight pulmonary irritation in rats (von Oettingen et al., 1942a). At 600 ppm (2260 mg/m^3) on the same dosing schedule, pulmonary irritation and renal casts in the collecting tubules were reported. Ungvary et al. (1980) reported decreased body weight and increased liver weight in female CFY rats exposed by inhalation at 1000 mg/m^3 for 6 hours/day, 5 days/week, for up to 6 months. No effects on hematologic parameters, body weight gain or tissue (lung, liver, kidney, heart, spleen) histology were observed in rats, guinea pigs, dogs or monkeys exposed to toluene at 107 ppm (389 mg/m^3) continuously for 90 days (Jenkins et al., 1970). Two rats exposed at this level died, however, during the exposure period. No indication was made regarding the cause of death in these animals, or whether treatment-related effects were exhibited.

In the only available chronic toxicity study reported to date, toluene was administered by inhalation to male and female F344 rats for 24 months at 30, 100 or 300 ppm (113, 377 or 1130 mg/m^3) for 6 hours/day, 5 days/week (CIIT, 1980). The only changes reported to be treatment-related were reduced hematocrit values in females exposed at 100 and 300 ppm and an increased corpuscular hemoglobin concentration in the high dose females.

These changes were not considered to be significant. Degenerative and inflammatory changes in various tissues were seen in both toluene-exposed and control animals.

Studies on workers exposed to toluene are relatively difficult to evaluate because exposure levels in almost all cases are not well defined and exposure often is to a combination of solvents. In general, the chronically or subchronically exposed workers exhibit CNS effects with reduced performance in psychomotor tests. Reduced performance was reported for a group of 100 automobile painters following a battery of intelligence and behavioral tests (Hanninen et al., 1976). Toluene exposure was determined to be ~30.6 ppm, while other solvents were detected at lower levels.

Case reports of abusers of toluene and toluene-containing compounds also indicate CNS changes as the primary effects. Boor and Hurtig (1977) reported ataxia, tremors, impairments in speech, hearing and vision, behavioral changes and impaired memory and concentration in an optician who used pure toluene (99%) as a cleaning solvent in a confined area. Exposure was frequent and of extended duration but toluene levels were not determined. As noted in Table VI-4, the signs and symptoms of others exposed to toluene by deliberate sniffing were, similarly, memory loss, behavioral changes, ataxia, incoordination and visual disorders. Exposure in most of these cases was at high levels, possibly approaching saturation (30,000 ppm), for short periods of time, repeated daily.

Hepatomegaly was noted in 13/61 airplane painters exposed to 100-1100 ppm toluene for periods up to 5 years (Greenburg et al., 1942). Altered enzyme levels (SGPT and SGOT) were reported in two groups of women exposed to 45 or 69 ppm toluene when compared with a control group of hospital nurses. Levels of SGOT and SGPT were, however, within a historical range (Shiojima et al., 1983).

Metabolic and renal tubular acidosis has been associated with long-term toluene abuse. Electrolyte imbalance, hypokalemia and hyperchloremia have apparently been caused by decreased hydrogen ion secretion in the distal convoluted tubules. As summarized in Table VI-7, several investigators have noted chemical urinary alterations in abusers of toluene, but no studies have shown renal effects in occupationally-exposed humans. The ~~NRC~~ NAS (1980) noted, however, that hypokalemia may lead to muscular weakness, a symptom previously associated with the CNS effects. Lethargy, ataxia and nausea also may be attributable to acidosis and renal effects.

Toluene was reported to cause embryonic mortality and fetotoxicity in CD1 mice (Nawrot and Staples, 1979). Pregnant mice were treated at 0.26, 0.43 or 0.87 g/kg/day by gavage on days 6-15 or 12-15 of gestation. A significant increase in embryo death was reported in all groups treated on days 6-15. Fetal weights were decreased in those groups treated at the two higher doses on days 6-15. Cleft palates were increased in fetuses of dams treated at 0.87 g/kg/day on days 6-15. Maternal toxicity was noted in mice treated on days 12-15 of gestation (dose levels not specified) but not in those treated on days 6-15 at any dose level. These data are available only in an abstract; a complete publication is not available for analysis.

Several teratogenicity studies have yielded largely negative results when toluene was administered by inhalation to rats and mice (Hudak and Ungvary, 1978; Litton Bionetics, Inc., 1981; Tatrai et al., 1980; Shigeta et al., 1982). The most prominent dose-related effect noted in these studies was evidence of delayed ossification and skeletal development. Rats treated at 1000 mg/m³ (8 hours/day on days 1-21 of gestation) or 1500 mg/m³ (24 hours/day on days 1-8 of gestation) produced fetuses with retarded skeletal development and poorly ossified sternebrae (Hudak and Ungvary, 1978). Tatrai et al. (1980) reported delayed skeletal ossification among fetuses exposed to 1000 mg toluene/m³ (24 hours/day) during days 7-14 of development. In mice, Hudak and Ungvary (1978) reported decreased fetal weights (evidence of embryotoxicity) in animals treated at 500 mg toluene/m³ (24 hours/day) on days 6-13 of development.

Quantification of Noncarcinogenic Effects

Derivation of 1-day HA. The effects of single inhalation exposure of humans to toluene for periods up to 8 hours have been reported by several investigators (von Oettingen et al., 1942a,b; Carpenter et al., 1944; Ogata et al., 1970; Gamberale and Hultengren, 1972). Based on the rather consistent dose-response data from a combination of these studies, it is evident that toluene at ~100 ppm for up to 8 hours/days causes no apparent adverse effects in humans. Subjective complaints of headache and drowsiness were reported by one volunteer exposed at 50 and 100 ppm, while consistent toluene-induced effects (fatigue, muscular weakness, incoordination) were evident in persons exposed to 200 ppm for 8 hours. Gamberale and Hultengren (1972) reported that a 20-minute exposure to 100 ppm toluene was a no-effect level when determined by perceptual speed and reaction time tests. At 200 ppm, toluene was noted as clearly causing toxic effects such as incoordination, exhilaration and prolonged reaction time (von Oettingen et al., 1942a,b; Carpenter et al., 1944; Ogata et al., 1970). These data substantiate the selection of 100 ppm (377 mg/m³) toluene as the NOAEL in humans exposed for up to 8 hours.

Using the NOAEL of 100 ppm (377 mg/m³) a 1-day HA would be derived as follows:

$$\text{TAD} = \frac{377\ \text{mg/m}^3 \times 20\ \text{m}^3\text{/day} \times 0.5 \times 8\ \text{hours/24 hours}}{70\ \text{kg}} = 17.9\ \text{mg/kg/day}$$

where:

TAD = total absorbed dose

377 mg/m³ = NOAEL, converted from 100 ppm exposure in humans (von Oettingen et al., 1942a,b)

8 hours/24 hours = duration of exposure in one day

20 m³/day = assumed daily ventilation volume for 70 kg adult

0.5 = estimated ratio of average dose absorbed (Nomiyama and Nomiyama, 1974a)

70 kg = weight of exposed individual

The 1-day HA for a 10 kg child is derived from this TAD as follows:

$$\text{1-day HA} = \frac{17.9\ \text{mg/kg/day} \times 10\ \text{kg}}{10 \times 1\ \ell\text{/day}} = 18\ \text{mg/}\ell$$

where:

17.9 mg/kg/day = total absorbed dose (TAD)

10 kg = weight of protected individual

10 = uncertainty factor, appropriate for use with human NOAEL, to accommodate for intraspecies sensitivity

1 ℓ = assumed volume of water consumed/day by 10 kg child

The proposed 1-day HA is 18 mg/ℓ for a 10 kg child.

Derivation of 10-Day HA. Studies appropriate for the derivation of the 10-day HA were not located in the available literature. It is therefore recommended that as a consevative estimate of exposure the 10-day HA for a 10 kg child utilize the RfD derived for the DWEL in the following calculation:

$$\text{10-day HA} = \frac{\text{RfD} \times 10\ \text{kg}}{1\ \ell/\text{day}} = \frac{0.3\ \text{mg/kg/day} \times 10\ \text{kg}}{1\ \ell/\text{day}} = 3\ \text{mg}/\ell$$

where:

RfD = 0.3 mg/kg/day

10 kg = assumed weight of exposed individual (child)

1 ℓ/day = assumed volume of water consumed/day by a 10 kg child

The proposed 10-day HA is 3 mg/ℓ.

Derivation of Longer-Term HA. Studies appropriate for the derivation of longer-term HAs were not located in the available literature. It is therefore recommended that the DWEL of 10 mg/ℓ be used as a conservative estimate of exposure for the longer-term HA for a 70 kg adult. The longer-term HA for a 10 kg child utilizes the RfD derived for the DWEL in the following derivation:

$$\begin{array}{c}\text{Longer-term HA}\\ \text{(child)}\end{array} = \frac{\text{RfD} \times 10\ \text{kg}}{1\ \ell/\text{day}} = \frac{0.3\ \text{mg/kg/day} \times 10\ \text{kg}}{1\ \ell/\text{day}} = 3\ \text{mg}/\ell$$

where:

RfD = 0.3 mg/kg/day

10 kg = assumed weight of exposed individual (child)

1 ℓ/day = assumed volume of water consumed/day by a 10 kg child

The longer-term value for a 10 kg child of 3 mg/ℓ is also a conservative estimate of exposure.

Assessment of Lifetime Exposure and Derivation of a DWEL. In the 6-month oral study conducted by Wolf et al. (1956), rats treated at 590 mg/kg/day, 5 days/week, showed no gross or histological signs of toxicity. Although toluene apparently was not administered at a level that was toxic, it will be assumed that this dose level is a conservative NOAEL in rats. CIIT (1980) conducted a 106-week study in which male and female F-344 rats were exposed by inhalation to toluene at 0, 30, 100 or 300 ppm (0, 113, 377 or 1130 mg/m^3) for 6 hours/day, 5 days/week. Results from this study have been audited and verified. No significant dose-related effects were identified. Although the final body weight of treated males were elevated significantly ($p<0.05$) and two hematologic parameters were significantly different from controls, the effects were not consistent throughout the study or between sexes, and were not regarded as treatment related.

Assuming, then, that 300 ppm (1130 mg/m^3) was the NOAEL in the CIIT (1980) study, an RfD can be derived as follows.

Step 1 - TAD Derivation

$$\text{TAD} = \frac{1130 \text{ mg/m}^3 \times 6 \text{ hours/24 hours} \times 20 \text{ m}^3\text{/day} \times 5/7 \times 0.5}{70 \text{ kg}} = 28.8 \text{ mg/kg/day}$$

where:

1130 mg/m^3 = NOAEL from rat data (CIIT, 1980)

6 hours/24 hours = exposure duration in one day

20 m^3/day = assumed daily respiratory volume of 70 kg adult

5/7 = conversion from 5 days/week dosing regimen to 7 days/week continuous exposure

0.5 = estimated ratio of average dose absorbed (Nomiyama and Nomiyama, 1974a)

70 kg = assumed weight of exposed individual (adult)

Step 2 - RfD Derivation

$$RfD = \frac{28.8 \text{ mg/kg/day}}{100} = 0.288 \text{ mg/kg/day } (0.3 \text{ mg/kg/day})$$

where:

28.8 mg/kg/day = total absorbed dose (TAD)

100 = uncertainty factor, appropriate for use with NOAEL from animal data

Step 3 - DWEL Derivation

$$DWEL = \frac{RfD \times 70}{2 \ \ell} = \frac{0.288 \text{ mg/kg/day} \times 70}{2 \ \ell\text{/day}} = 10 \text{ mg/}\ell$$

where:

0.3 mg/kg/day = RfD
70 = assumed weight of exposed individual
2 ℓ/day = assumed volume of water consumed/day by 70 kg adult

The proposed DWEL based upon the chronic inhalation study in rats (CIIT, 1980) is 10 mg/ℓ. This DWEL is supported by data from a subchronic oral NOAEL of 590 mg/kg/day in rats (Wolf et al., 1956). The DWEL of 10 mg/ℓ is based only upon exposure to toluene in the drinking water and does not account for exposure in food or by other routes of exposure.

Carcinogenic Effects

The chronic (106-week) bioassay of toluene in F344 rats of both sexes reported no carcinogenic effects (CIIT, 1980). Gross and microscopic examination of tissues and organs identified no increase in neoplastic tissue or tumor masses among rats treated at 30, 100 or 300 ppm when compared with controls. This bioassay was inadequate since the highest dose administered (300 ppm) was well below the MTD. Also, only one species/strain was tested.

Prechronic carcinogenicity testing of commercial toluene administered by gavage to rats (F344) and mice (B6C3F1) has been conducted, but a technical report on the data has not been issued (NCI, 1983). The NCI (1983) also has started a chronic bioassay of commercial toluene in rats and mice exposed by inhalation. Testing is in progress (quality assessment in progress as of January 13, 1987), so neither preliminary nor final data are available. An updated assessment of the carcinogenic potential of toluene should await the completion of these tests.

Quantification of Carcinogenic Effects

An estimate of cancer risk cannot be determined because of lack of data on the carcinogenic potential of toluene. Applying the criteria described in EPA's guidelines for assessment of carcinogenic risk (U.S. EPA, 1986b), toluene may be classified as Group D: Not classifiable as to human carcinogenicity. This classification reflects the inadequate nature of the available data thus preventing the development of a more definite statement regarding carcinogenic potential.

Existing Guidelines, Recommendations and Standards

An ambient water quality criterion level of 14.3 mg/ℓ has been derived by the U.S. EPA (1980). This level is based upon the subchronic, oral NOEL of 590 mg/kg/day in rats reported by Wolf et al. (1956). An uncertainty factor of 1000 was used to derive an RfD for humans of 30 mg/day. The criterion considers toluene exposure through consumption of water and contaminated fish or shellfish.

The FDA has approved toluene for use in products intended for food packaging such as paper coatings (Code of Federal Regulations, 1983a), cellophane (residue limit of 0.1%) (Code of Federal Regulations, 1983b) and acrylic plastics (Code of Federal Regulations, 1983c). Toluene use as a solvent in pesticide formulations on growing crops is exempt from limitation (Code of Federal Regulations, 1982).

As previously noted, the TLV for toluene in the air is 100 ppm for an 8-hour TWA concentration; a 15-minute exposure limit of 150 ppm also is recommended (ACGIH, 1982). Absorption through the skin also may contribute to overall toluene exposure and is considered in these limits. OSHA (1981) recommends 200 ppm as an 8-hour TWA with an acceptable ceiling concentration of 300 ppm. The maximum peak concentration is 500 ppm toluene for a 10-minute exposure. NIOSH (1973) recommended 100 ppm toluene as the 8-hour TWA and 200 ppm as a maximum level.

Special Groups at Risk

Interactions with Other Chemicals. As reviewed in the Synergistic/Antagonistic Effects Section in Chapter VII, several organic solvents have been determined to affect toluene metabolism and toxicity. Studies in rats indicate that benzene and toluene may be metabolized by similar enzyme systems. Concomitant exposure, therefore, may lead to mutual metabolic inhibition. Co-administration of benzene and toluene results in prolonged excretion of metabolites of both compounds, indicating reciprocal inhibition (Ikeda et al., 1972). Metabolic inhibition was evident only when benzene and toluene were administered at higher levels (390 mg/kg/day) suggesting hepatic enzyme saturation (Sato and Nakajima, 1979b). The problem of increased toxicity from delayed excretion of toluene or benzene was not addressed in these studies. In humans, exposure to benzene (25 ppm) and toluene (100 ppm) at their respective TLV levels had no inhibitory effect on metabolism or excretion of either compound (Sato and Nakajima, 1979b).

m-Xylene, co-administered with toluene in rats, also caused a delay in the excretion of toluene metabolites (Ogata and Fujii, 1979). Other compounds that have been reported to inhibit toluene excretion when both were administered simultaneously are trichloroethylene (Ikeda, 1974), perchloroethylene (Smyth et al., 1969a) and ethanol (Hudak et al., 1978). Morvai and Ungvary (1979) reported that toluene and ethanol exposure acted additively on increasing vascular resistance in the myocardium.

In human volunteers exposed to toluene at 80 ppm by inhalation, subsequent ethanol consumption at ~500 mg/kg caused an increase (~40%) in blood-toluene levels (Waldron et al., 1983). Toluene metabolism apparently was inhibited by ethanol. These authors additionally noted that blood toluene levels were lowest in men who consumed ethanol regularly. It was suggested that both toluene and ethanol are metabolized by alcohol dehydrogenase and that regular ethanol consumption induces this enzyme and can cause more rapid toluene metabolism (Waldron et al., 1983).

Recently, toluene was reported to potentiate the maternal and embryo toxicity of aspirin in rats (Ungvary et al., 1983). Co-administration of toluene and aspirin also increased the incidence of skeletal abnormalities in exposed fetuses ($p<0.01$).

High Risk Subpopulations. Three groups generally are considered to be at increased risk from exposure to toxic levels of toluene (see the section on High Risk Subpopulations in Chapter VI). Toluene-exposed workers, abusers, smokers, and pregnant women and their developing fetuses are at highest risk of developing adverse health responses as a result of toluene exposure.

Workers who are exposed to toluene on the job represent a great number of people at elevated risk of toluene toxicity. The use of toluene is ubiquitous; thus, workers from a wide range of industries and occupations may be exposed, both acutely and chronically. As noted in the section on High Risk Subpopulations in Chapter VI, workers may be exposed during toluene refinement or during production and use of the multitude of toluene-containing compounds. Most occupational exposures to toluene are through its use as a solvent and usually involve simultaneous exposure to other organic solvents such as xylene, benzene or various alcohols. The synergistic and inhibitory interactions summarized in the Existing Criteria and Standards Section in this chapter suggest that workers exposed to toluene and other common solvents (xylene and hexane) simultaneously may be at higher or lower risk (depending on type of solvents involved) than those exposed to a single chemical. Regular consumption of ethanol increases the rate of toluene metabolism, probably by enzyme induction, while intermittent alcohol consumption may interfere with toluene metabolism.

People who smoke also may be at increased risk of exposure to toluene. Since a smoker may absorb ~0.1 mg toluene/cigarette, this represents a major source of toluene exposure in humans. Heavy smokers who also are occupationally exposed to toluene may represent the group at highest risk.

As is quite often the case in determining groups at high risk to chemical toxicity, pregnant women and, more specifically, their fetuses, are often of concern. Because of the physiological nature of the placenta and the delayed development of metabolic enzymes in the fetal liver, chemical exposure of the mother can affect the fetus. The available teratogenicity studies in laboratory animals (see the section on Teratogenicity in Chapter V) indicate that embryotoxicity and embryo death may occur when maternal toxicity is not exhibited. In an abstract, Nawrot and Staples (1979) reported that oral administration of toluene at all doses tested (0.26, 0.43 and 0.87 g/kg/day on days 6-15 of gestation) increased embryo death. Delayed skeletal ossification and low fetal weights were reported by Hudak and Ungvary (1978) in rats and mice exposed to toluene vapor at 1500 mg/m^3 (24 hours/day on days 1-8 of gestation) or 500 mg/m^3 (24 hours/day on days 6-13), respectively. There are no reports available that correlate toluene exposure in humans (see the section on Subchronic and Chronic Exposure in Chapter VI) to any effects on fetal death or development, but based on animal data, the fetus and pregnant mother should be regarded as being at increased risk.

Summary

Based on the available data on noncarcinogenic effects of toluene on laboratory animals and humans, 1- and 10-day HAs and an DWEL for toluene have been derived (Table VIII-1). The 1-day HAs for a child is 18 mg/ℓ. This level is based upon the human NOAEL of 100 ppm (377 mg/m^3) toluene for 8 hours by inhalation.

TABLE VIII-1

Summary of Data Used to Derive HA and DWEL

Criteria	Dose	Duration	Effect	Value of HA or DWEL		Reference
				Adult	Child	
1-day HA	17.9 mg/kg/day (377 mg/m³ x 8 hours/24 hours x 20 m³/day x 0.5 ÷ 70 kg)	8 hours	human NOAEL		10 mg/ℓ	von Oettingen et al., 1942a,b
10-day HA	0.3 mg/kg/day	2 years	rat NOAEL, inhalation		3 mg/ℓ*	CIIT, 1980
Longer-Term HA	0.3 mg/kg/day	2 years	rat NOAEL, inhalation	10 mg/ℓ*	3 mg/ℓ*	CIIT, 1980
DWEL	28.8 mg/kg/day (1130 mg/m³ x 20 m³/day x 6 hours/day x 5 days/7 days x 0.5 ÷ 70 kg)	2 years	rat NOAEL, inhalation	10 mg/ℓ	NA	CIIT, 1980

*The 10-day and longer-term HAs are more conservative estimates of exposure derived from the DWEL.

NA - Not applicable

Appropriate studies for the derivation of the 10-day and longer-term HAs were not located in the available literature. Therefore, the RfD will be used to derive the 10-day and longer-term HA for a 10 kg child as a more conservative estimate of exposure. The recommended 10 day and longer-term HA for a 10 kg child is 3 mg/ℓ. The DWEL of 10 mg/ℓ was adopted as a conservative estimate of exposure for the adult longer-term HA.

The DWEL was based upon the most conservative RfD determined from data on rats treated by inhalation (300 ppm, 6 hours/day, 5 days/week) for 2 years (CIIT, 1980). The DWEL derived from these data was 10 mg/ℓ.

Toluene has not been tested adequately for carcinogenicity, and, therefore, quantification of carcinogenic effects cannot be performed. Carcinogenicity bioassays, involving inhalation exposures in rats and mice, are underway in the National Toxicology Program, but no data are available as yet.

Multiple exposures to toluene and other organic solvents may result in inhibited or delayed metabolism and excretion of toluene. Potentially, this could increase the toxicity of toluene. Millions of workers are exposed to toluene, making them a large increased-risk group. Many are exposed to toluene and other solvents simultaneously. Cigarette smokers also are exposed to increased levels of toluene in the smoke. Workers exposed to toluene who also smoke may be at higher risk. Although data from human exposure are inadequate, some animal studies suggest that fetuses may be at increased risk to toluene toxicity.

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